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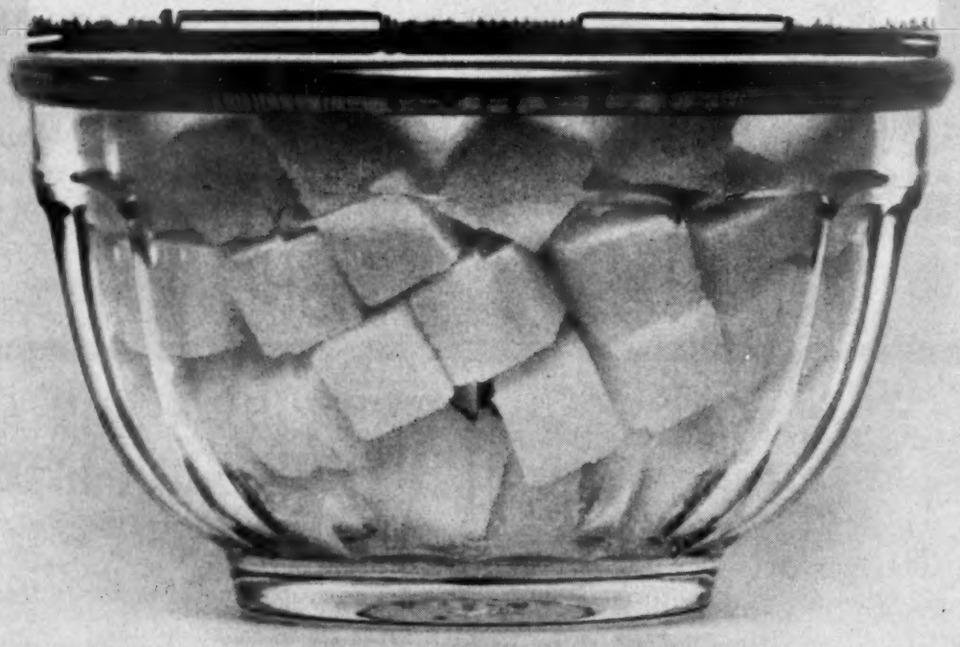

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(1) Danowski, T. S.: Diabetes Mellitus, Baltimore, Williams & Wilkins, 1957, p. 239. (2) McCune, W. G.: M. Clin. North America 44:1479, 1960. (3) Ackerman, R. F., et al.: Diabetes 7:398, 1958.

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The American Journal of Medicine

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Clinical Studies

A Third Type of Periodic Paralysis, with Normokalemia and Favourable Response to Sodium Chloride . . . . DAVID C. POSKANZER AND DAVID N. S. KERR 328

This interesting report describes a family afflicted with periodic paralysis not associated with hypokalemia, indeed worsened by administration of potassium salts, and also differing in significant respects from adynamia episodica hereditaria. Designated sodium responsive, normokalemic periodic paralysis, the disorder is inherited as an autosomal dominant with apparently complete penetrance and becomes clinically manifest in the first decade of life. The periodic attacks which in the severe form include quadriplegia, are brought on particularly by unwontedly prolonged inactivity such as oversleeping or sitting through a double feature in the movies (irrespective of the quality of the films). The attacks can be prevented or mitigated by large sodium chloride intake or measures which facilitate sodium retention. The precise metabolic nature of the anomaly is obscure.

Relationship of Multiple Endocrine Adenomas to the Syndrome of Ulcerogenic Islet Cell Adenomas (Zollinger-Ellison). Occurrence of Both Syndromes in One Family . . . . JAKOB R. SCHMID, ALEXIS LABHART AND P. H. ROSSIER 343

The authors describe a remarkable family of seven siblings of which three sisters had multiple endocrine adenomas involving pituitary, pancreas and/or parathyroids; one brother had hyperparathyroidism; and two brothers died of atypical peptic ulcers doubtless due to ulcerogenic islet cell adenomas. This study, then, provides additional evidence that the Zollinger-Ellison syndrome may occur as part of the more inclusive, genetically transmitted entity of multiple endocrine adenomatosis.

Myxedema Following Radioactive Iodine Therapy of Hyperthyroidism
ROBERT L. SEGAL, SOLOMON SILVER, STEPHEN B. YOHALEM AND SERGEI FEITELBERG 354

In a series of 1,603 patients with hyperthyroidism treated with radioactive iodine and followed thereafter for years, in 112 (7 per cent) myxedema developed, requiring continuous thyroid replacement therapy, and in 103 more transient myxedemia developed. It is evident that to obtain remission of hyperthyroidism in a satisfactory percentage of cases, myxedema will be produced in some instances, due to difficulties in gauging dosage, but it is also clear that the incidence can be reduced

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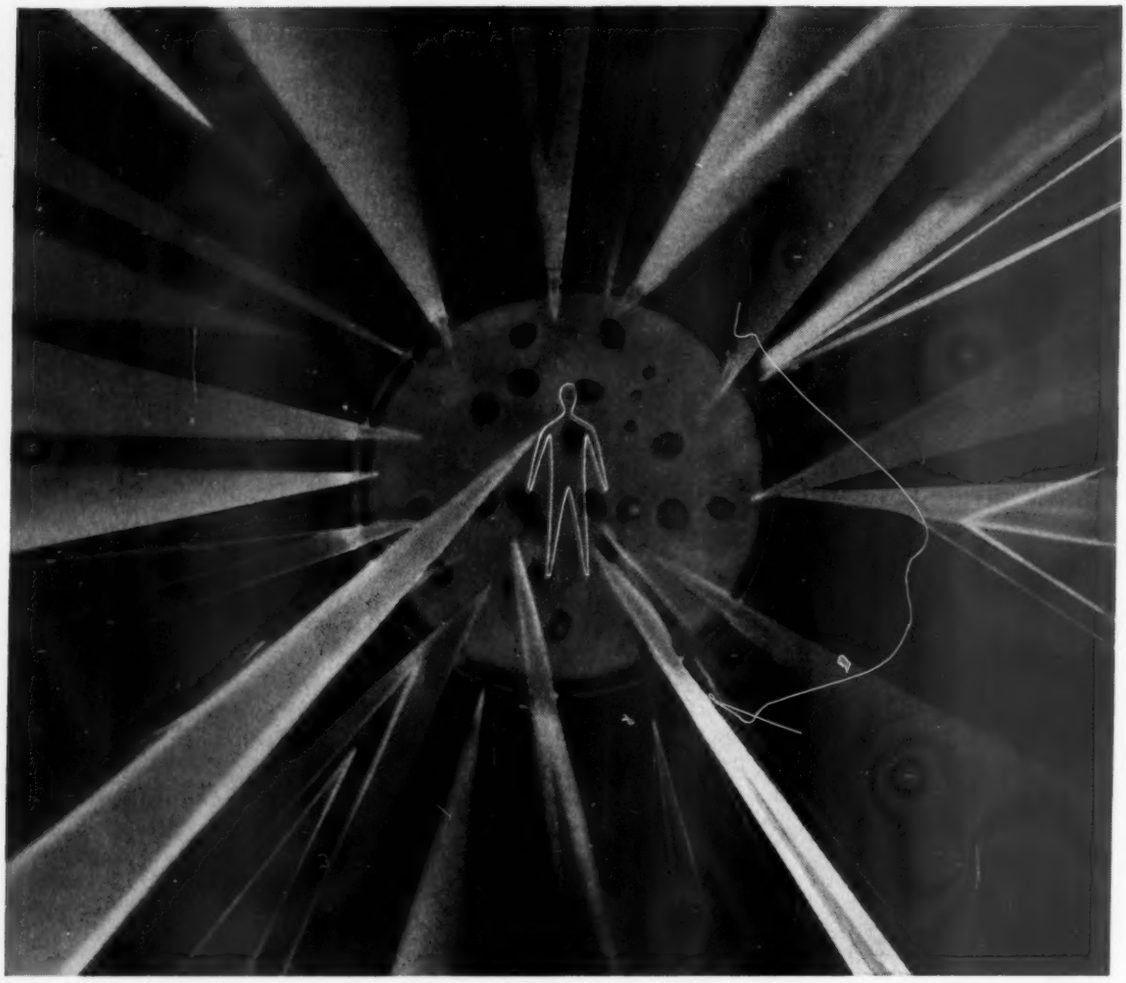

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to a low figure by awareness of the factors that predispose to iatrogenic myxedema. In this series it was found that age of the patient, prior therapy with other modalities, size of the thyroid gland, acuteness of onset of hyperthyroidism and radiosensitivity of the thyroid tissue all played a role in overshooting or undershooting the dosage mark. Patients with multinodular goiters with hyperthyroidism seemed to respond as well as those with diffuse goiters and gave no higher incidence of myxedema.

Bacterial Infection and Some Effects of Chemoprophylaxis in Chronic Pulmonary Emphysema. I. Chemoprophylaxis with Intermittent Tetracycline
ANNE L. DAVIS, EVELYN J. GROBOW, RALPH TOMPSETT AND JOHN H. MCCLEMENT 365

The frequency with which recurrent bronchitis aggravates the course of idiopathic pulmonary emphysema has led to widespread use of antibiotics therapeutically and prophylactically. The latter use has been largely empiric although there are some conflicting reports about its merit. In the present long-term study, the authors employed tetracycline two days weekly. While a statistically significant reduction in the number of infections of the lower respiratory tract occurred in the treated group, the infection rate was so high that the effect of prophylaxis was minimal. Subjective improvement was even less prominent. No change in pulmonary function occurred in either treated or control group. Bacteriologic studies failed to demonstrate any importance of H. influenzae as a factor in the acute bronchitic episodes.

A Non-Atheromatous Proliferative Vascular Lesion of the Retina in Diabetes Mellitus. Role in the Etiology of Diabetic Retinopathy
HERMAN T. BLUMENTHAL, MORRIS ALEX AND SIDNEY GOLDENBERG 382

In addition to the well known inflammatory, atheromatous, thrombotic and hypertensive changes in the walls of the retinal vessels in patients with diabetes, proliferative lesions occur, in considerably greater frequency than in non-diabetic subjects. These latter lesions, described in detail in the present study, are believed by the authors to have a special significance for the vascular complications of diabetes, in the eye and elsewhere. Speculating upon this relationship, the authors suggest that an immunologic mechanism may be the key both to the proliferative retinal vessels described and, indeed, to the vascular complications of diabetes generally.

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Recent Progress in Human Chromosome Analysis and Its Relation to the Sex Chromatin . . . . . ARTHUR R. SOHVAL 397

This comprehensive review of cytogenetics will serve admirably as an introduction to an area of investigation which already has proved to be exceedingly productive. Beginning with a much needed glossary, Dr. Sohval goes on to describe the genetic mechanisms causing chromosomal abnormalities, such as translocation, non-disjunction and mosaicism. He then considers the karyotypes characterizing hermaphroditism, Klinefelter's syndrome, Turner's syndrome, mongolism and various multiple congenital abnormalities, touching also upon the as yet fragmentary information concerning the leukemias. There is a final section on the origin and significance of sex chromatins.

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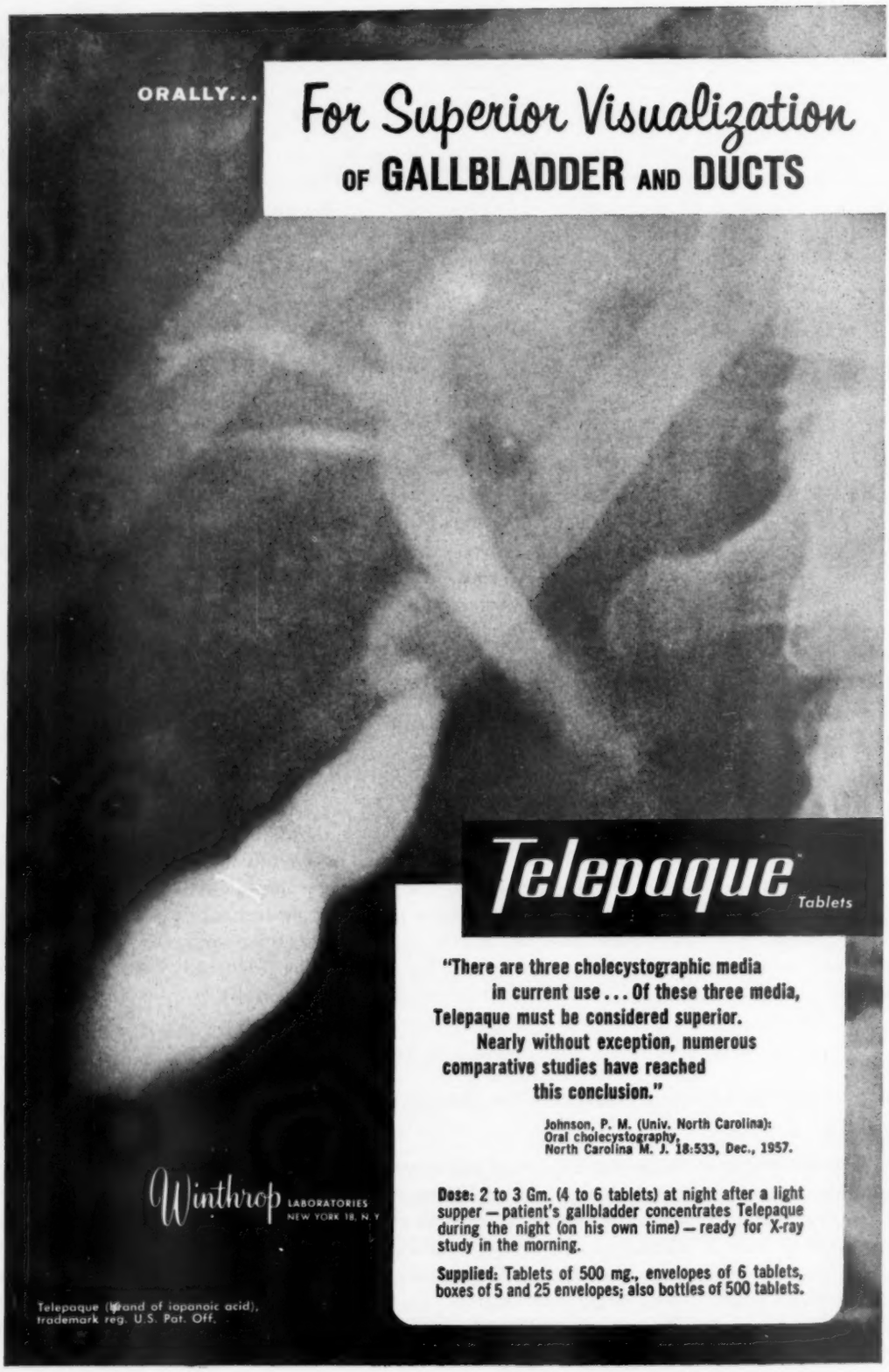
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Chromosomal Aberrations in Human Disease. A Review of the Status of Cytogenetics in Medicine . . . . . KURT HIRSCHHORN AND HERBERT L. COOPER 442

Cytogenetics has recently come rapidly to the fore in the study of the mechanisms of human disease as it is already evident that chromosomal aberrations play an important role in the pathogenesis of certain disorders. Notable among these is mongolism (trisomy of the twenty-first autosome), Klinefelter's syndrome (XXY instead of the normal XY male sex chromosomes), and Turner's syndrome (XO instead of the normal female XX). How such chromosomal errors come about in the process of meiosis or mitosis is lucidly explained in this review, which serves admirably as an introduction to this new and rewarding area of exploration of disease.

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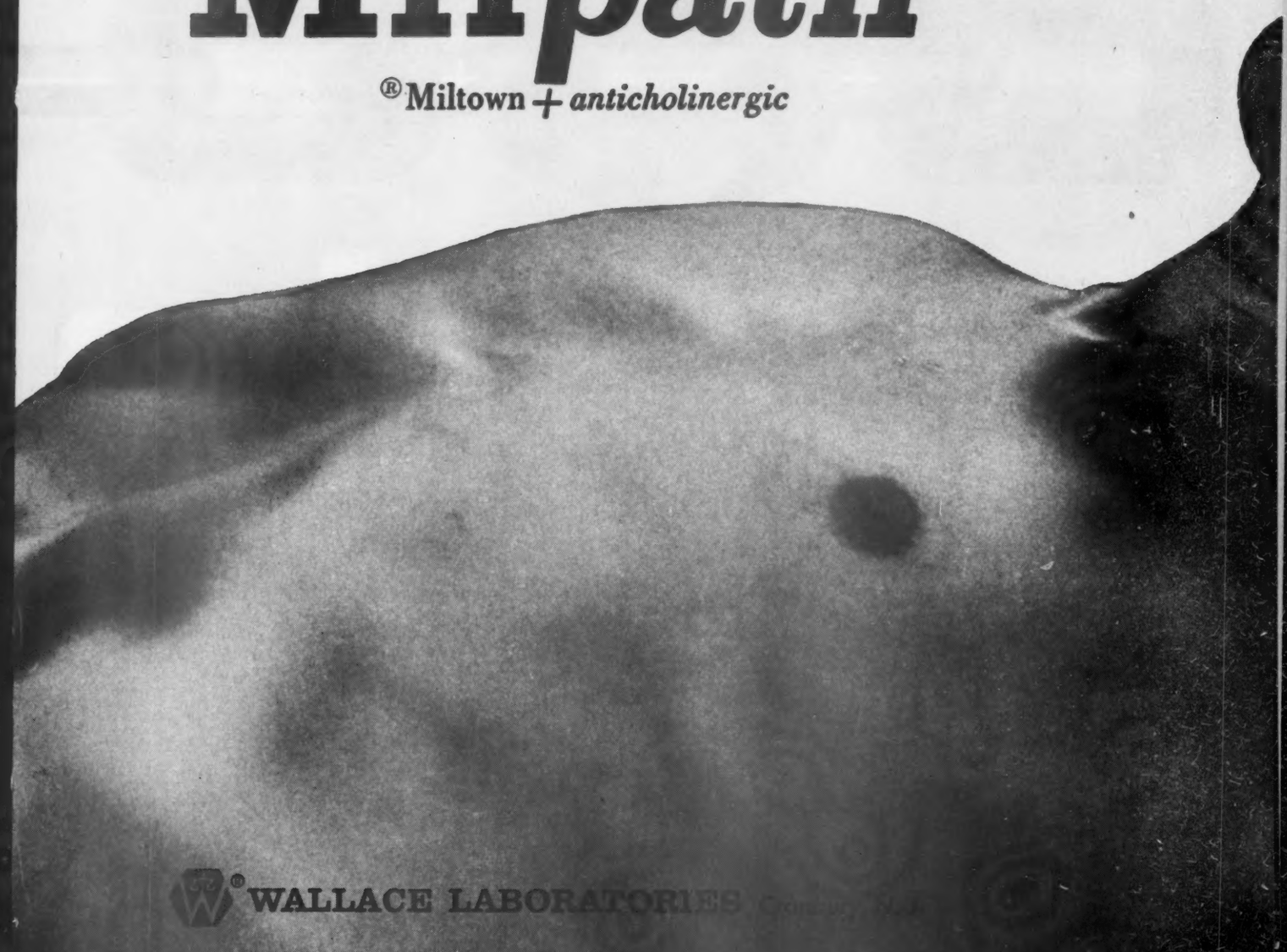

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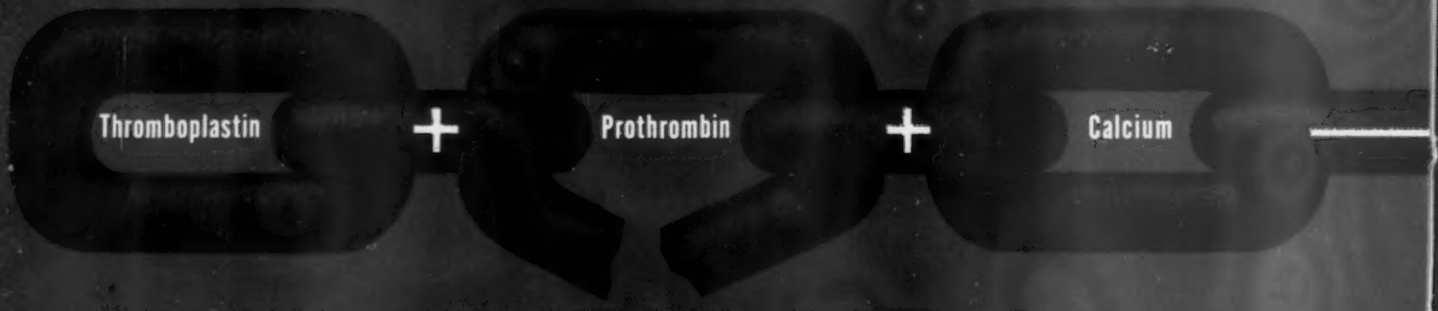

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these two arthritics and an ulcerative

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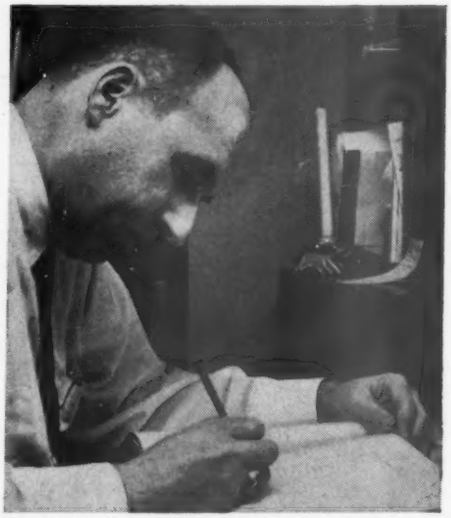
Suffering from chronic ulcerative colitis since 1953, D. G. was placed on METICORTEN 5 mg. t.i.d. in 1955. In the following year, because of the co-



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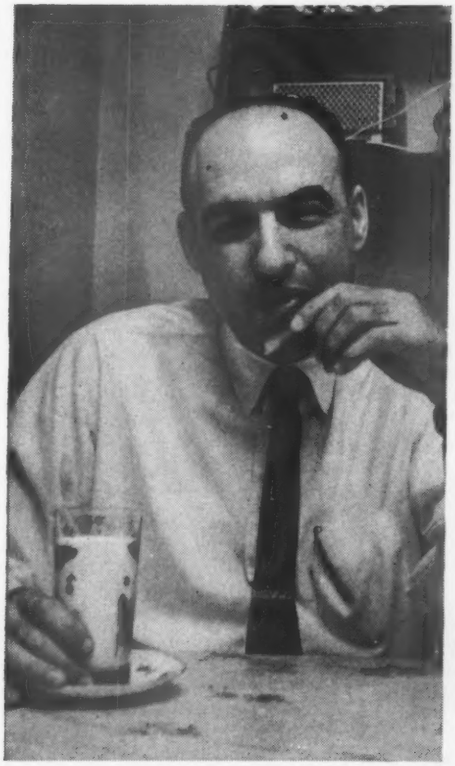

litis and arthritis of the finger joints, knee and elbows, the dosage was increased to 10 mg. t.i.d. His arthritic symptoms disappeared during 1956. From 1956 to 1960, he was on a dosage of 5 mg. t.i.d. His colitis improved. Now off METICORTEN, he feels well, has one formed stool daily. Rectal and sigmoid mucosa are normal. At no time were there any side reactions from the METICORTEN.

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Case histories of J. G. and H.M., courtesy of Joel Goldman, M.D., Johnstown, Pa. Case history of D. G., courtesy of Asher Winkelstein, M.D., New York City. Photographs of these three patients were taken at their respective homes or places of work.

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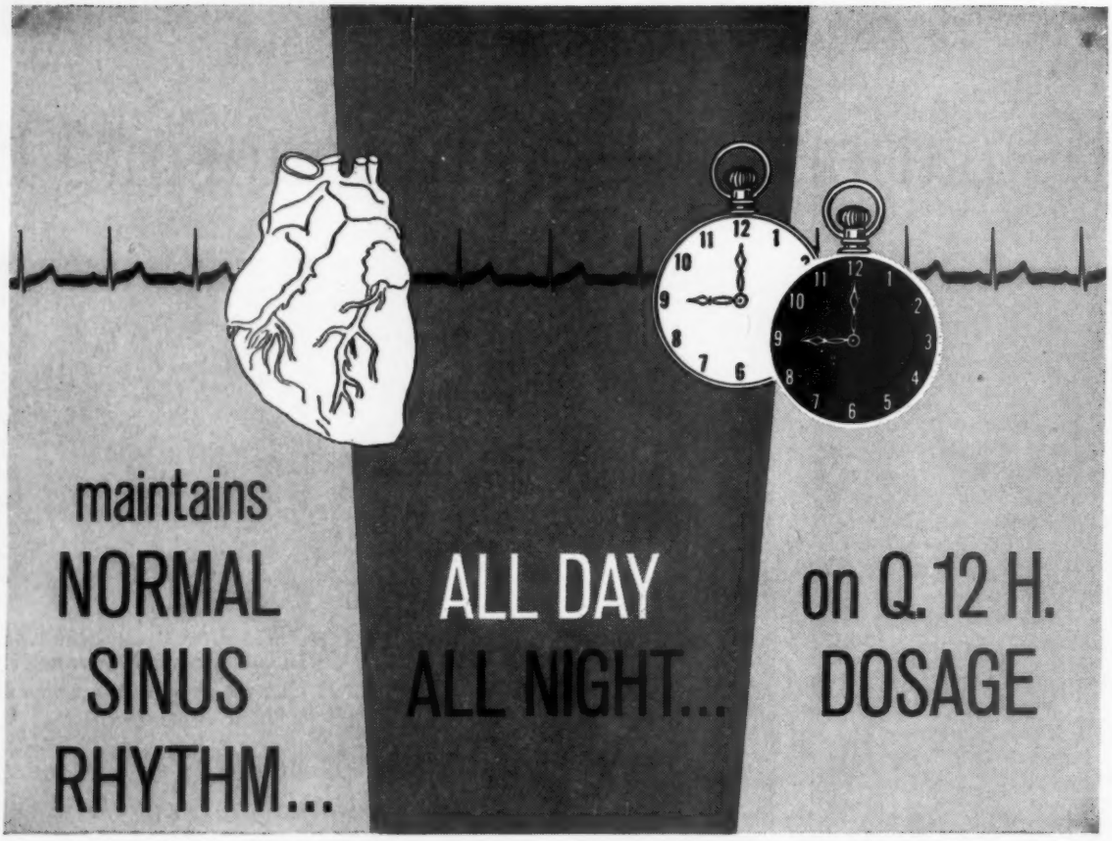

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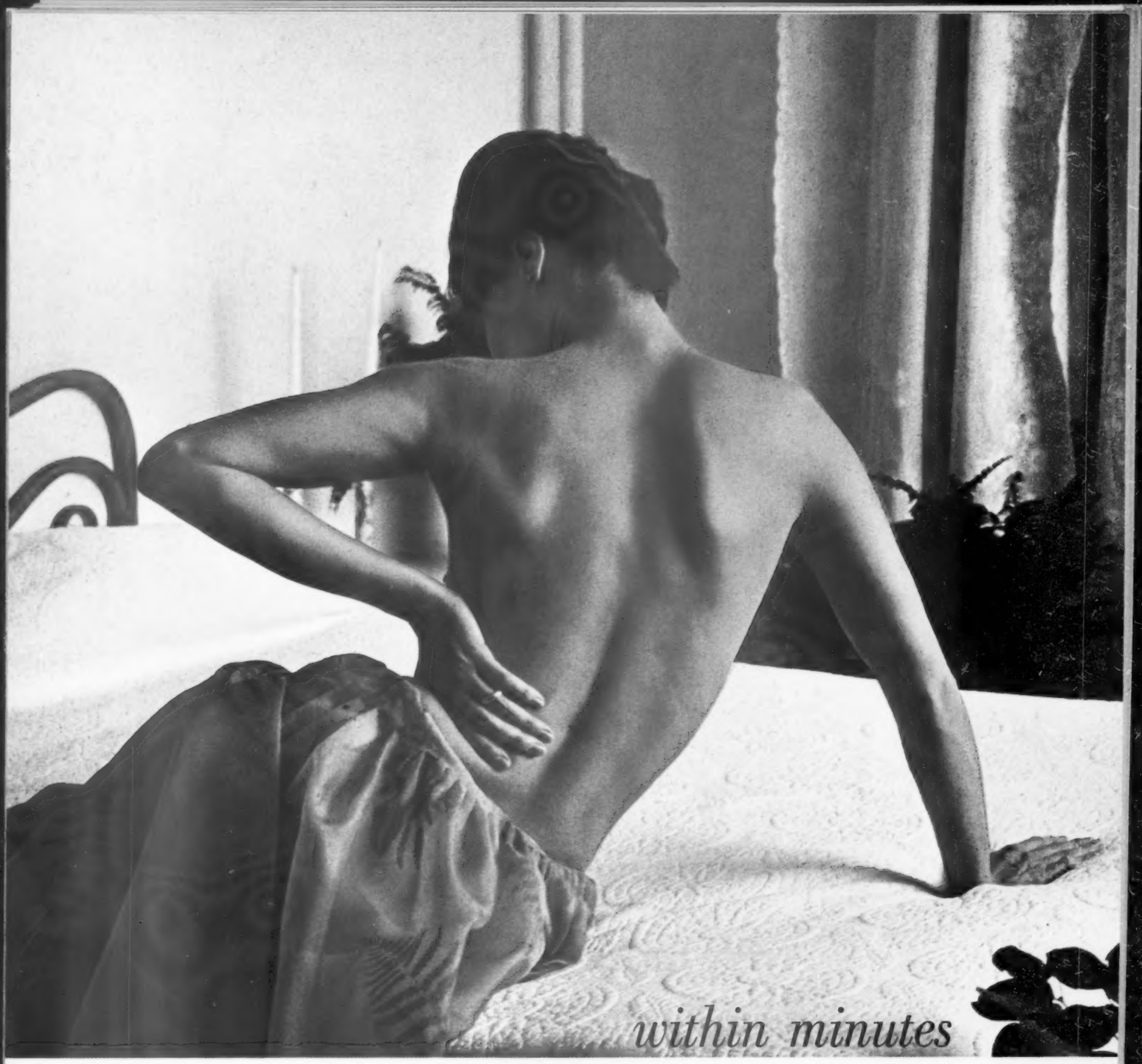

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SUPPLY: ROBAXIN Tablets, 0.5 Gm. (white, scored) in bottles of 50 and 500. ROBAXIN Injectable, each ampul containing 1.0 Gm. of methocarbamol in 10 cc. of sterile solution.

A. H. ROBINS CO., INC., Richmond 20, Virginia

Making today's medicines with integrity . . . seeking tomorrow's with persistence

When the pursuit of success
N 700

leads to visceral distress...

restore normal smooth muscle function through dependable autonomic sedation

The uniformly dependable antispasmodic-sedative action of DONNATAL relieves hypermotility, hypertonicity and spasticity of smooth muscle **at all levels** of the gastrointestinal tract: pharynx, esophagus, stomach, small intestine and large intestine.

Donnatal incorporates **natural** belladonna alkaloids in optimal synergistic ratio, supplemented by phenobarbital in low dosage, for concurrent control of both somatogenic and psychogenic factors.

For dosage flexibility–

DONNATAL® TABLETS
CAPSULES
ELIXIR

Antispasmodic maintenance under a t.i.d. dosage regimen

For prolonged effects–

DONNATAL® EXTENTABS®

All-day or all-night spasmolytic benefits on a single dose, equal to the effect of one DONNATAL tablet uniformly sustained for 10 to 12 hours.

	In each Tablet, Capsule, or 5 cc. Elixir	In each Extentab
Hyoscyamine sulfate	0.1037 mg.	0.3111 mg.
Atropine sulfate	0.0194 mg.	0.0582 mg.
Hyoscine hydrobromide	0.0065 mg.	0.0195 mg.
Phenobarbital	(¼ gr.) 16.2 mg.	(¾ gr.) 48.6 mg.

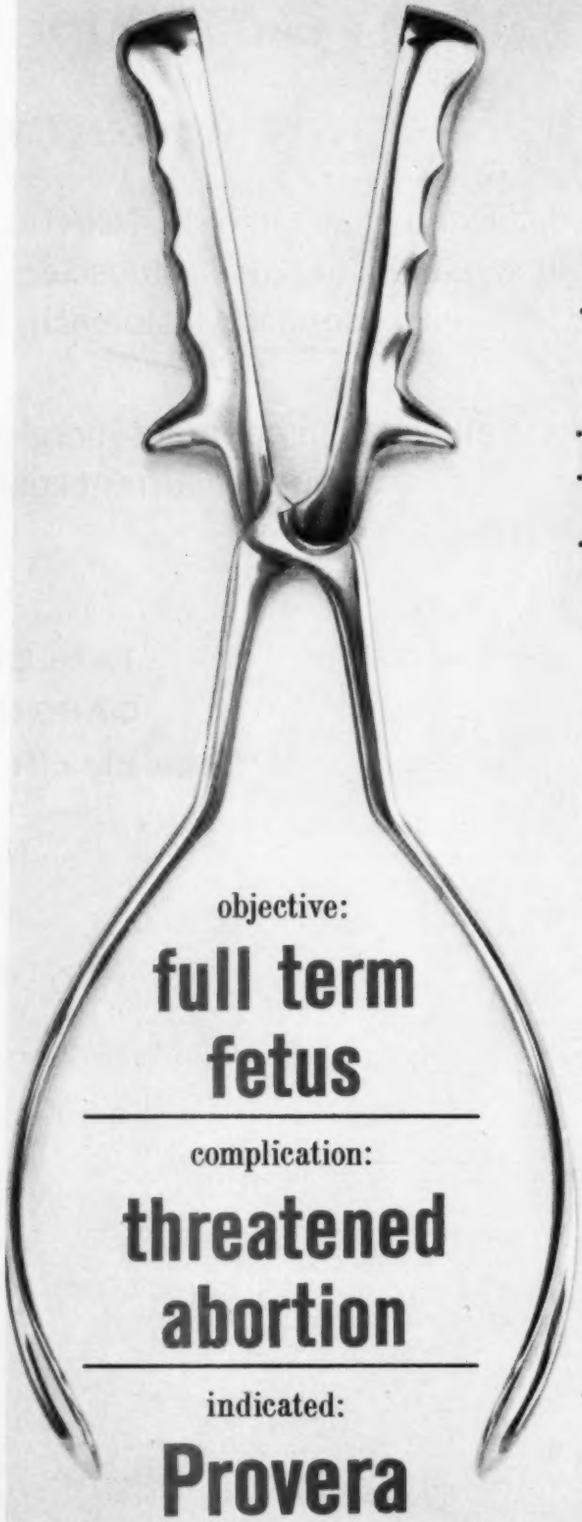
DONNATAL® Robins

natural belladonna alkaloids with phenobarbital

Prescribed by more physicians than any other antispasmodic

A. H. ROBINS CO., INC.
RICHMOND 20, VIRGINIA

Making today's medicines with integrity... seeking tomorrow's with persistence



objective:

full term fetus

complication:

threatened abortion

indicated:

Provera

Upjohn

Here are four reasons why:

- Provera is the only commercially—available oral progestational agent that will maintain pregnancy in critical tests in ovariectomized animals.
- No significant side effects have been encountered.
- It is available for both oral and parenteral administration.
- Provera gives the economy of effective action from small doses.

Brief Basic Information

	◐ Oral Provera*	⟶ I.M. Depo-Provera**
Description	Upjohn brand of medroxyprogesterone acetate.	Aqueous suspension, 50 mg. Provera per cc., for intramuscular injection only.
Indications	Threatened and habitual abortion, infertility, secondary amenorrhea, functional uterine bleeding.	Threatened and habitual abortion.
Dosage Threatened abortion	10 to 30 mg. daily until acute symptoms subside.	50 mg. I. M. daily while symptoms are present, followed by 50 mg. weekly through 1st trimester, or until fetal viability is evident.
Habitual abortion		
1st trim.	10 mg. daily.	50 mg. I.M. weekly.
2nd trim.	20 mg. daily.	100 mg. I.M. q. 2 wks.
3rd trim.	40 mg. daily, through 8th month.	100 mg. I.M. q. 2 wks. through 8th month.
Supplied	2.5 mg. scored, pink tablets, bottles of 25; 10 mg. scored, white tablets, bottles of 25 and 100.	Sterile aqueous suspension for intramuscular use only. 50 mg. per cc., in 1 cc. and 5 cc. vials.†

Precautions: Clinically, Provera is well tolerated. No significant untoward effects have been reported. Animal studies show that Provera possesses adrenocorticoid-like activity. While such adrenocorticoid action has not been observed in human subjects, patients receiving large doses of Provera continuously for prolonged periods should be observed closely. Likewise, large doses of Provera have been found to produce some instances of female fetal masculinization in animals. Although this has not occurred in human beings, the possibility of such an effect, particularly with large doses over a long period of time, should be considered.

Provera, administered alone or in combination with estrogens, should not be employed in patients with abnormal uterine bleeding until a definite diagnosis has been established and the possibility of genital malignancy has been eliminated.

†Each cc. of Depo-Provera contains: Medroxyprogesterone acetate, 50 mg.; Polyethylene glycol 4000, 28.8 mg.; Polysorbate 80, 1.92 mg.; Sodium chloride, 8.65 mg.; Methylparaben, 1.73 mg.; Propylparaben, 0.19 mg.; Water for injection, q.s.

The Upjohn Company, Kalamazoo, Michigan

*TRADEMARK, REG. U.S. PAT. OFF. **TRADEMARK

CAPLA™
(mebutamate, Wallace)
CENTRAL ACTING PRESSURE LOWERING AGENT

ANNOUNCING

CAPLA™

A new drug that works in a new way to control blood pressure without serious side effects

CAPLA™

(mebutamate, Wallace)

CENTRAL ACTING PRESSURE LOWERING AGENT

Capla acts centrally at the brainstem vasomotor center

Reduces blood pressure by central action is not a ganglionic blocker

Capla is a new *kind* of drug to treat hypertension. Chemically, Capla is 2-methyl-2-*sec*-butyl-1, 3-propanediol dicarbamate. It is unrelated chemically to any other antihypertensive agent. Capla does not block ganglia, reduce blood volume or interfere with neurohormonal balance.

New therapy for hypertension

Because of its action at the brainstem vasomotor control center, Capla is a new therapy for hypertension. It is effective alone in the treatment of mild to moderate hypertension, and can be combined with diuretics or peripherally acting antihypertensives in more severe cases.

Exceptionally well tolerated

Capla acts rapidly, producing substantial blood pressure reduction within two hours, yet it does not produce postural hypotension. It has proved exceptionally well tolerated in clinical use and has no known contraindications. Capla has not produced changes in renal, hematological, hepatic or endocrine function. It is rapidly eliminated and has no cumulative effects.

CAPLA™

(mebutamate, Wallace)

CENTRAL ACTING PRESSURE LOWERING AGENT

Controls blood pressure without serious side effects

Capla does not produce depression, postural hypotension, nasal congestion or gastric hyperacidity

Capla helps minimize one of the most difficult problems of hypertension therapy – unwanted and often serious side effects.

With Capla you have effective therapy without the unpleasant side effects which often cause patients to abandon treatment.

Side effects with Capla, when they do occur, are mild and usually transient. Transient drowsiness sometimes occurs, usually at higher dosage.

Mild calming effect

Patients on Capla often report a mild calming effect. This effect, together with the unusual freedom from serious side effects, makes therapy gratifying for both the patient and the physician.

Compatible with other drugs

Hypertensive patients with other disorders can receive Capla along with other medications.

For example, patients with congestive heart failure, angina, and diabetes mellitus can receive Capla along with such medications as digitalis, nitrates, and insulin—without aggravating these other disorders.

CAPLA™

(mebutamate, Wallace)

CENTRAL ACTING PRESSURE LOWERING AGENT

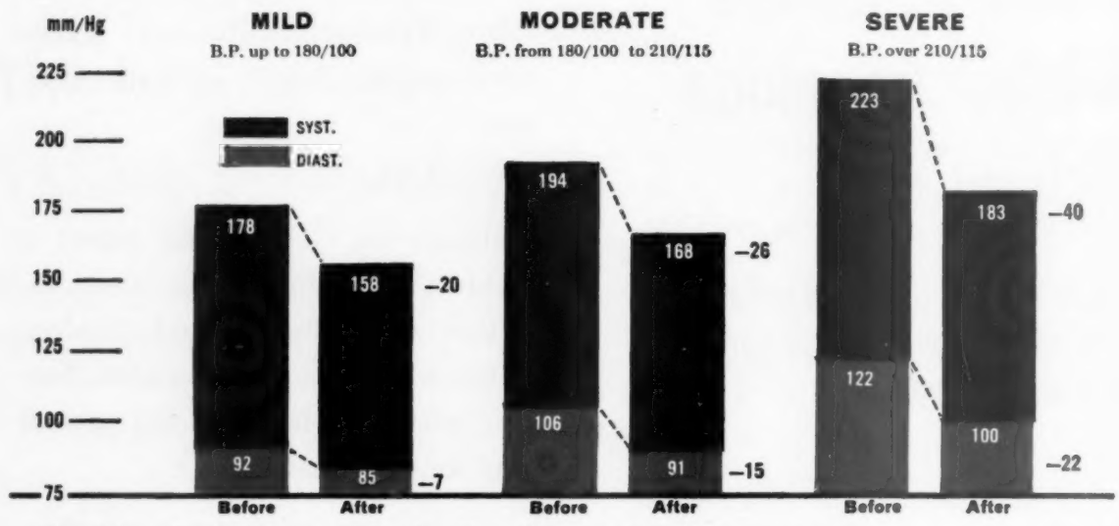
Lowers blood pressure effectively in clinical use

CLINICAL & PHARMACOLOGICAL REPORTS

1. Berger, F. M., and Margolin, S : A Centrally Acting Blood Pressure Lowering Agent (W-583). Fed. Proc. 20:113 (March) 1961. 2. Diamond, S., and Schwartz, M. Scientific Exhibit at Ill. State Med. Soc. Chicago, (May) 1961. 3. Douglas, J. F., Ludwig, B. J.,, Ginsberg, T. and Berger, F M.: Studies on W-583 Metabolism. Fed. Proc. 20:113 (March) 1961. 4. Duarte, C., Brest, A. N., Kodama, R., Naso, F., and Moyer, J. H : Observations on the Antihypertensive Effectiveness of a New Propanediol Dicarbamate (W-583). Curr. Ther. Res., 2:148-52 (May) 1960 5. DuChez, J. W., Scientific Exhibit at Amer. Academy of Gen Practice, Miami, (April) 1961. 6. Kletzkin, M., and Berger, F. M.: A Centrally Acting Antipressor agent. Fed Proc. 20:113 (March) 1961. 7. Mulinos, M. G., Scientific Exhibit at Amer. Coll. Card New York, (May) 1961 8. Mulinos, M. G., Saltefors, S., Boyd, L. J. and Cronk, G. A.: Human Pharmacology Studies with W-583. Fed. Proc. 20:113 (March) 1961. 9. Shubin, H., Scientific Exhibit, Amer. Coll. Card. New York, (May) 1961.

Average Reductions In Systolic And Diastolic Blood Pressure Reported With Capla

(325 patients)



Usual dose, Capla 300 mg., q.i.d.—duration of therapy, 3 weeks to over 1 year.

These data show that Capla reduces both systolic and diastolic blood pressure, usually in proportion to initial pre-treatment elevations.

CAPLA™

CENTRAL ACTING PRESSURE LOWERING AGENT

Wallace Laboratories
Cranbury, New Jersey

DOSAGE: the recommended dose of Capla is one 300 mg. tablet three or four times daily, before meals and at bedtime. The dosage should be adjusted to individual requirements; for example, older patients may require lower dosage.

COMPOSITION: each white, scored tablet contains 300 mg. of Capla (mebutamate, Wallace).

SUPPLIED: bottles of 100, scored tablets.

Literature and samples to physicians on request.

Menopausal distress: a syndrome involving all three levels of the autonomic nervous system

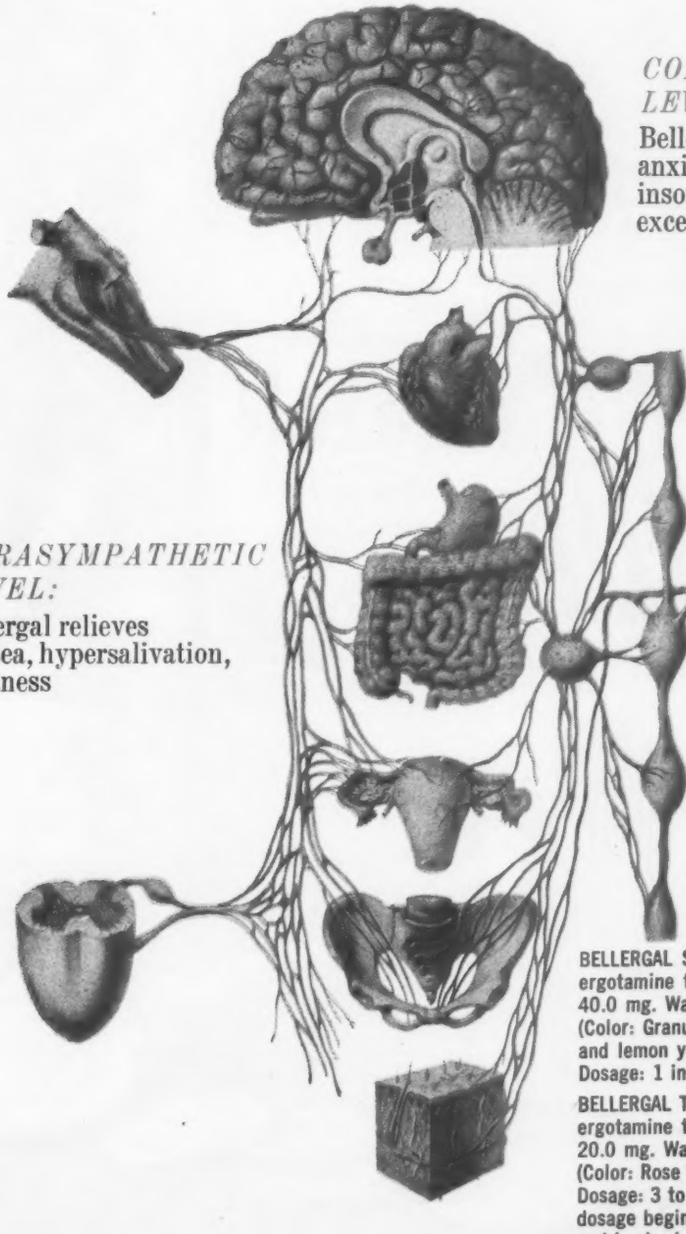
for functional disorders of the menopause

Bellergal®

SPACETABS®

stabilizes the entire autonomic nervous system

(*without disturbing endocrine balance*)



CORTICAL LEVEL:
Bellergal relieves anxiety, irritability, insomnia, headache, excessive fatigability

SYMPATHETIC LEVEL:
Bellergal relieves hot flashes, palpitations, tachycardia, tremor, sweats

PARASYMPATHETIC LEVEL:
Bellergal relieves nausea, hypersalivation, faintness

SANDOZ

BELLERGAL SPACETABS—Bellafoline 0.2 mg., ergotamine tartrate 0.6 mg., phenobarbital 40.0 mg. Warning: May be habit forming. (Color: Granular pattern of green, apricot and lemon yellow; compressed.) Dosage: 1 in the morning, and 1 in the evening.

BELLERGAL TABLETS—Bellafoline 0.1 mg., ergotamine tartrate 0.3 mg., phenobarbital 20.0 mg. Warning: May be habit forming. (Color: Rose beige, sugar-coated.) Dosage: 3 to 4 daily. In more resistant cases, dosage begins with 6 tablets daily and is slowly reduced.

Now! 2 appetizing foods make serum cholesterol control easier, more effective than ever!

Your Patients Can Enjoy Fine Foods With Minimum Diet Changes!

For good eating while maintaining serum cholesterol control

Leading authorities agree that where reduction of serum cholesterol levels is indicated, fat intake should not exceed ⅓ of total calories and of this, at least ⅓ should be polyunsaturated fats.

Polyunsaturated fats, such as those found in corn oil, are rich in the linoleates which are important in reducing serum cholesterol levels. This has been proven time and again in nutritional studies of hypercholesterolemia. Mazola Margarine and Mazola Corn Oil have outstanding P/S (polyunsaturate to saturate) ratios. Thus the hypercholesterolemic patient can usually enjoy the same appetizing foods as the rest of the family.

Mazola Corn Oil is unexcelled in polyunsaturates and lowest in saturates of all leading brands of vegetable oils. Mazola's P/S ratio is far higher than that of any other leading food oil. Your patient will find Mazola Corn Oil ideally suited for salad dressings and frying; also for baking wherever *liquid* shortenings are called for in the recipe.

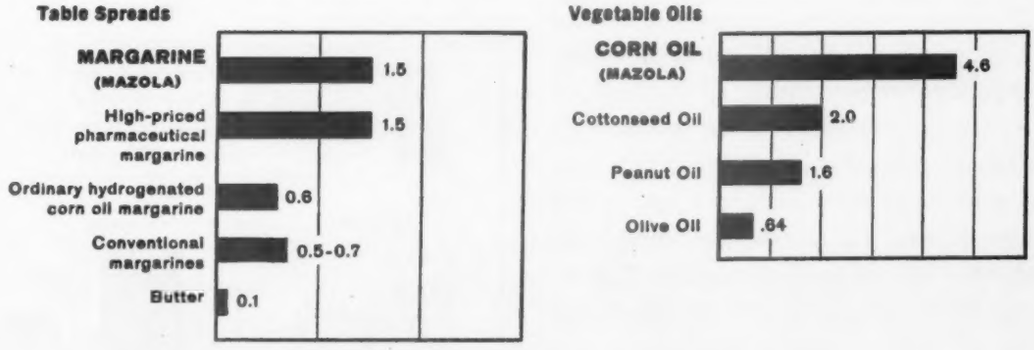
Mazola Margarine contains *liquid* Mazola Corn Oil as a major ingredient. This corn oil is not hydrogenated, thereby preserving its rich content of linoleates. Mazola Margarine contains 2 to 3 times as much natural linoleates as any other margarine readily available in grocery stores from coast to coast. Its taste, color and handling characteristics are unexcelled.

AVERAGE COMPOSITIONS OF MAZOLA® MARGARINE AND MAZOLA® CORN OIL
(All figures are in grams.)

	MAZOLA MARGARINE		MAZOLA CORN OIL	
	100 grams	2 oz. (4 tbsp.)	100 grams	1 fl. oz. (2 tbsp.)
Fatty Acids				
Polyunsaturated	21	12	51	14
Monounsaturated	40	23	32	9
Saturated	14	8	11	3
Natural Sitosterols	0.5	0.3	1	0.3
Natural Tocopherols	0.08	0.045	0.08	0.020
Cholesterol	none	none	none	none
Sodium	0.9	0.5	none	none

MAZOLA MARGARINE—410 Calories/2 oz.; Iodine Value—96
MAZOLA CORN OIL—250 Calories/fl. oz.; Iodine Value—124

RATIO OF POLYUNSATURATES/SATURATES
(Average values.)



CORN PRODUCTS COMPANY 10 East 56th Street, New York 22, N.Y.

No. 1 **of a series reporting on Endo Laboratories' Anticoagulant Survey**

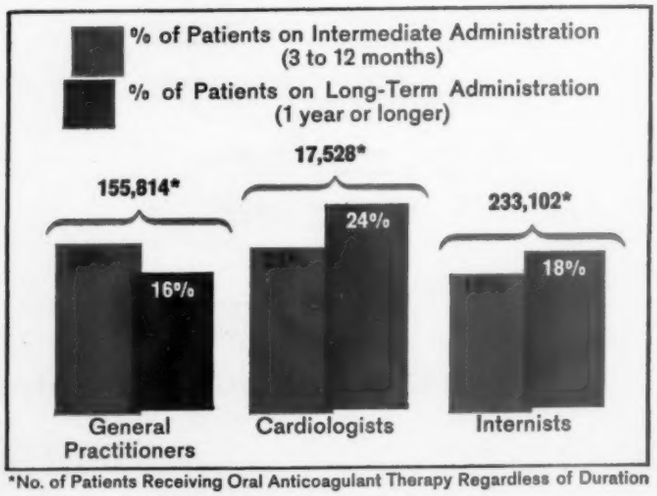
Nationwide Survey Explores Current Practices in Anticoagulant Therapy

Early in 1961 Endo Laboratories undertook a comprehensive survey of physicians' attitudes toward oral anticoagulants and their application in daily practice. Approximately 90,000 physicians received a questionnaire which covered such basic matters as *when, how,* and *for what periods* anticoagulants were used, the *specific advantages* of the agent most often selected by general practitioners, internists, and cardiologists, *factors* relating to the effective, safe administration of anticoagulants, and other pertinent areas of clinical interest. A total of 10,016 physicians completed and returned the *Anticoagulant Survey.*

The vast accumulation of data has now been analyzed and tabulated so that one can draw conclusions regarding the actual practice of thousands of physicians. Endo is happy to present the findings of *Anticoagulant Survey* in the first of a series of brief reports. It is our purpose thereby to contribute to a better understanding of the use of anticoagulants, therapeutically and prophylactically. We would greatly appreciate your comments and observations on this series. For more detailed information on *Anticoagulant Survey,* write to the Professional Service Dept., Endo Laboratories, Richmond Hill 18, New York.

Long-Term Anticoagulant Therapy of Myocardial Infarction More Widely Accepted

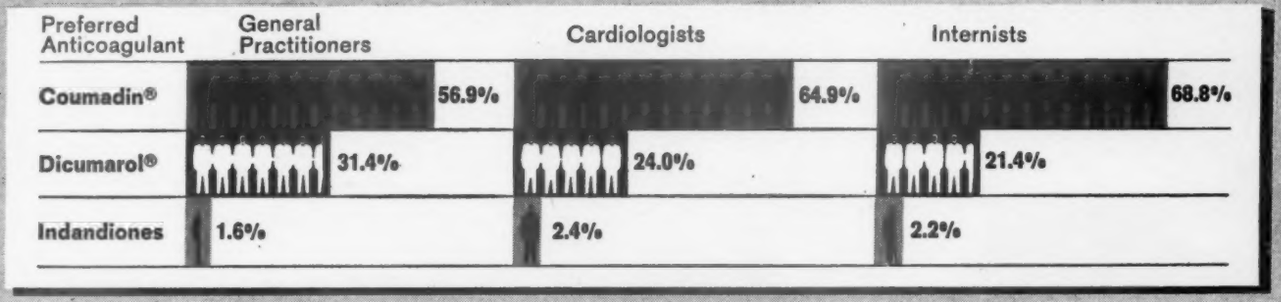
Broad clinical experience[1-12] has indicated the value of oral anticoagulants in preventing or minimizing the occurrence of myocardial infarctions and extending life expectancy, particularly on long-term use. Reports from general practitioners and specialists reveal that these life-saving agents are being used *prophylactically* not only after frank myocardial infarction but also in *impending* infarction associated with angina pectoris. The following table shows the percentage of patients who have been maintained on oral anticoagulant therapy for extended periods of time, according to replies of physicians who answered the Endo anticoagulant questionnaire.



*No. of Patients Receiving Oral Anticoagulant Therapy Regardless of Duration

Specialists and General Practitioners Using Coumadin Increasingly

A comparison of Coumadin (warfarin sodium), Dicumarol (bishydroxycoumarin), and Indandiones showed that coumarin derivatives were prescribed most often and that Coumadin was the drug of choice by a wide margin.



It is noteworthy that 80% of the general practitioners, 80% of the internists, and 86% of the cardiologists favoring Coumadin reported their use of oral anticoagulants to be **increasing.** Of 3,092 responding physicians in general practice, 564 had had patients on Coumadin for one year, 628 for two years, 479 for three years, 215 for four years, 181 for five years, and 149 for **more than five years.**

Specialists Favor Tapering Anticoagulant Dosage Before Discontinuance

Abrupt cessation of anticoagulant therapy, especially after long-term administration, has been cautioned against by some clinicians[5,12] because of the possibility of a consequent state of *hypercoagulability* with increased risk of thromboembolism. *Anticoagulant Survey* showed that of the general practitioners who stated their procedure more than half did *not* taper the dose before discontinuance; on the other hand, a majority of internists and cardiologists did taper the dose over several weeks.

Periodic Prothrombin Time Tests Essential to Effectiveness and Safety

Especially in long-term therapy, *periodic prothrombin time determinations* are recognized as vital to effectiveness and safety. Most of the reporting physicians are having these tests performed at one-week, two-week, or four-week intervals. The following data from *Anticoagulant Survey* indicate that the predominant trend is toward testing at intervals of two weeks or longer, and that this procedure is observed by a significantly higher percentage of reporting physicians who prefer Coumadin than by those who favor Dicumarol.

Preferred Anticoagulant	PT Tests Every Week		PT Tests Every 2 to 4 Weeks or Longer	
	GP's	Specialists	GP's	Specialists
Coumadin	17%	16%	63%	67%
Dicumarol	27%	23%	55%	60%

The preferred prothrombin time range was between 1½ to 2½ times normal control; the most favored prothrombin time was twice normal, or 26 seconds. (Complications of anticoagulant therapy will be discussed in a subsequent report.)

Physicians Stress Predictability and Ease of Maintenance in Selecting Anticoagulant

In characterizing the *advantages* of the oral anticoagulant most often prescribed (Coumadin), general practitioners, cardiologists, and internists were unanimous in listing, in order of importance: (1) *more predictable effect*, (2) *easier maintenance*, and (3) *single daily dose*. Among Dicumarol prescribers, on the other hand, "single daily dose" was first in importance, "more predictable effect" second, and "easier maintenance" third.

These data confirm the unusually consistent recognition of Coumadin advantages which have made possible a smoother, more easily managed long-term anticoagulant regimen. Since Coumadin may be given I.M. and I.V. as well as orally, it is also the most versatile of anticoagulants in hospital or office practice.

References: 1. Thomes, A. B., et al.: J.A.M.A. 176:181, 1961. 2. Nora, J. J.: Ibid. 174:118, 1960. 3. Idem: M. Times 89:502, 1961. 4. Beamish, R. E., and Storrie, V. M.: Heart Bull. 10:41, 1961. 5. Nichol, E. S., et al.: Am. Heart J. 55:142, 1958. 6. Manchester, B.: Ann. Int. Med. 47:1202, 1957. 7. Report of Working Party on Anticoagulant Therapy in Coronary Thrombosis to M. Res. Council: Brit. M. J. 1:803, 1959. 8. Friedberg, C. K.: New York J. Med. 58:877, 1958. 9. Seaman, A. J.: GP 22:135, No. 4, 1960. 10. Stephens, C. A. L., Jr.: Arizona Med. 17:499, 1960. 11. Canady, E. W., et al.: Illinois M. J. 113:50, 1958. 12. Littman, M. L.; Barrett, E. A., and Shapiro, S.: Scientific Exhibit, 110th Annual Meet., A.M.A., New York, N. Y., June 25-30, 1961.

Another professional service of Endo Laboratories Makers of

COUMADIN®

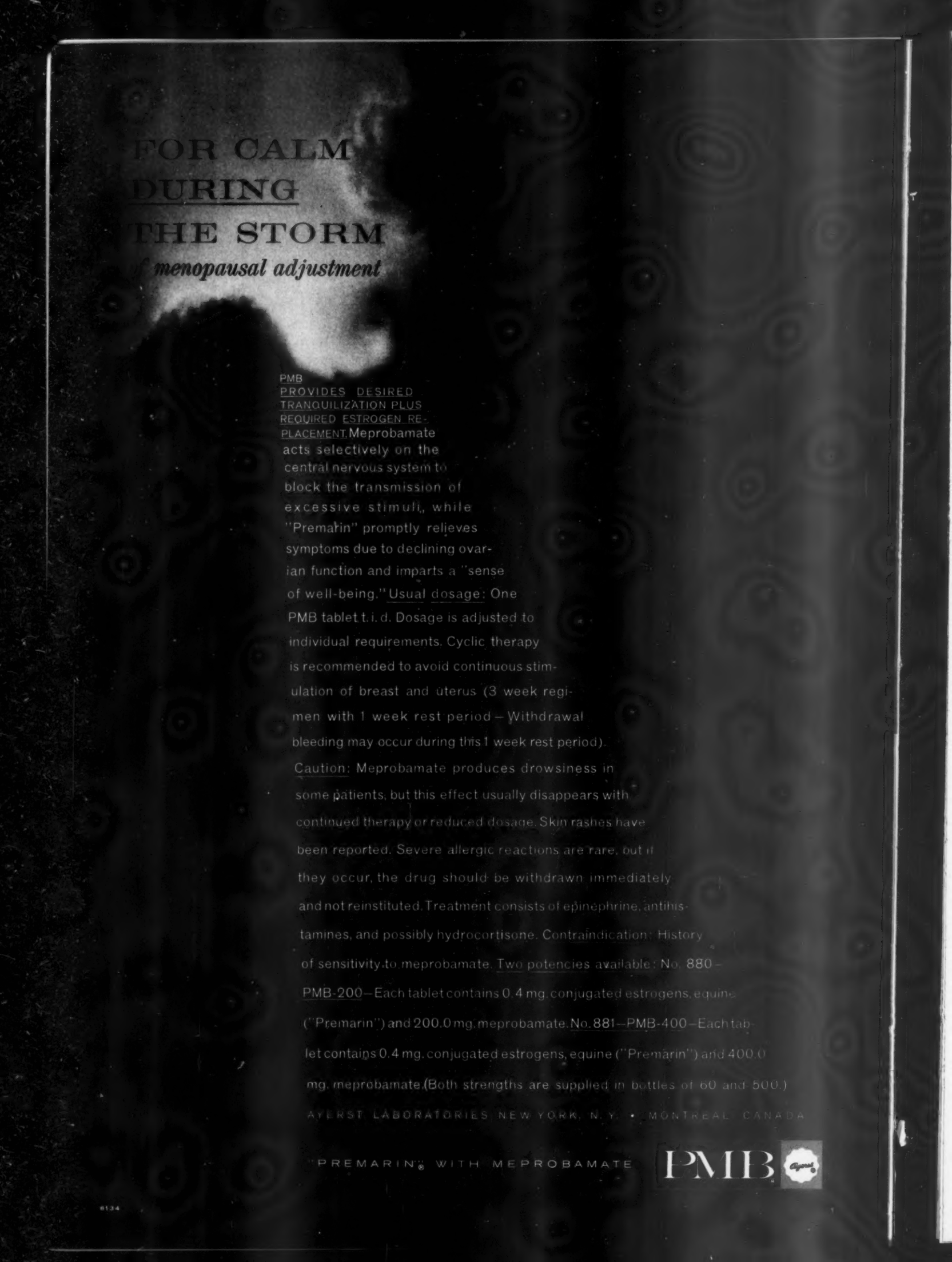
the proven anticoagulant for long-term maintenance

FOR ORAL, INTRAVENOUS OR INTRAMUSCULAR USE

Coumadin (warfarin sodium) is manufactured under license from the Wisconsin Alumni Research Foundation, and is supplied as scored tablets of 2, 5, 7½, 10, and 25 mg. as well as in 50 mg. and 75 mg. single-injection units.

Endo®

ENDO LABORATORIES • Richmond Hill 18, New York



PMB PROVIDES DESIRED TRANQUILIZATION PLUS REQUIRED ESTROGEN REPLACEMENT. Meprobamate acts selectively on the central nervous system to block the transmission of excessive stimuli, while "Premarin" promptly relieves symptoms due to declining ovarian function and imparts a "sense of well-being." Usual dosage: One PMB tablet t. i. d. Dosage is adjusted to individual requirements. Cyclic therapy is recommended to avoid continuous stimulation of breast and uterus (3 week regimen with 1 week rest period – Withdrawal bleeding may occur during this 1 week rest period). Caution: Meprobamate produces drowsiness in some patients, but this effect usually disappears with continued therapy or reduced dosage. Skin rashes have been reported. Severe allergic reactions are rare, but if they occur, the drug should be withdrawn immediately and not reinstituted. Treatment consists of epinephrine, antihistamines, and possibly hydrocortisone. Contraindication: History of sensitivity to meprobamate. Two potencies available: No. 880–PMB-200–Each tablet contains 0.4 mg. conjugated estrogens, equine ("Premarin") and 200.0 mg. meprobamate. No. 881–PMB-400–Each tablet contains 0.4 mg. conjugated estrogens, equine ("Premarin") and 400.0 mg. meprobamate. (Both strengths are supplied in bottles of 60 and 500.)

AYERST LABORATORIES NEW YORK, N.Y. • MONTREAL CANADA

"PREMARIN"® WITH MEPROBAMATE

PMB®

6134

Hypertension and congestive failure controlled with Serpasil®-Esidrix®

Photographs used with permission of the patient.

Mr. H.V., a 61-year-old retired pharmacist with hypertensive arteriosclerotic heart disease, was hospitalized in 1957 after a myocardial infarction. Blood pressure at this time ranged from 176/100 to 184/106 mm. Hg. The patient had associated congestive failure with ankle edema and dyspnea.

Serpasil-Esidrix Tablets #1 were added to the existing regimen of digitalis and low-salt diet in April, 1959. In the first 6 weeks of treatment, blood pressure decreased steadily to a range of 156/80 to 166/84 mm. Hg. Examination at the end of 6 weeks revealed no evidence of congestive failure. Neck veins were no longer distended; ankle edema was not present.

Mr. V.'s blood pressure is now stabilized at a satisfactory level and he has had no side effects from Serpasil-Esidrix. He can climb stairs without shortness of breath; he gets around more easily and feels better generally.

Serpasil-Esidrix combines in one tablet the antihypertensive and calming effects of Serpasil with the diuretic and anti-hypertensive-potentiating actions of Esidrix—for control of high blood pressure plus many complications.

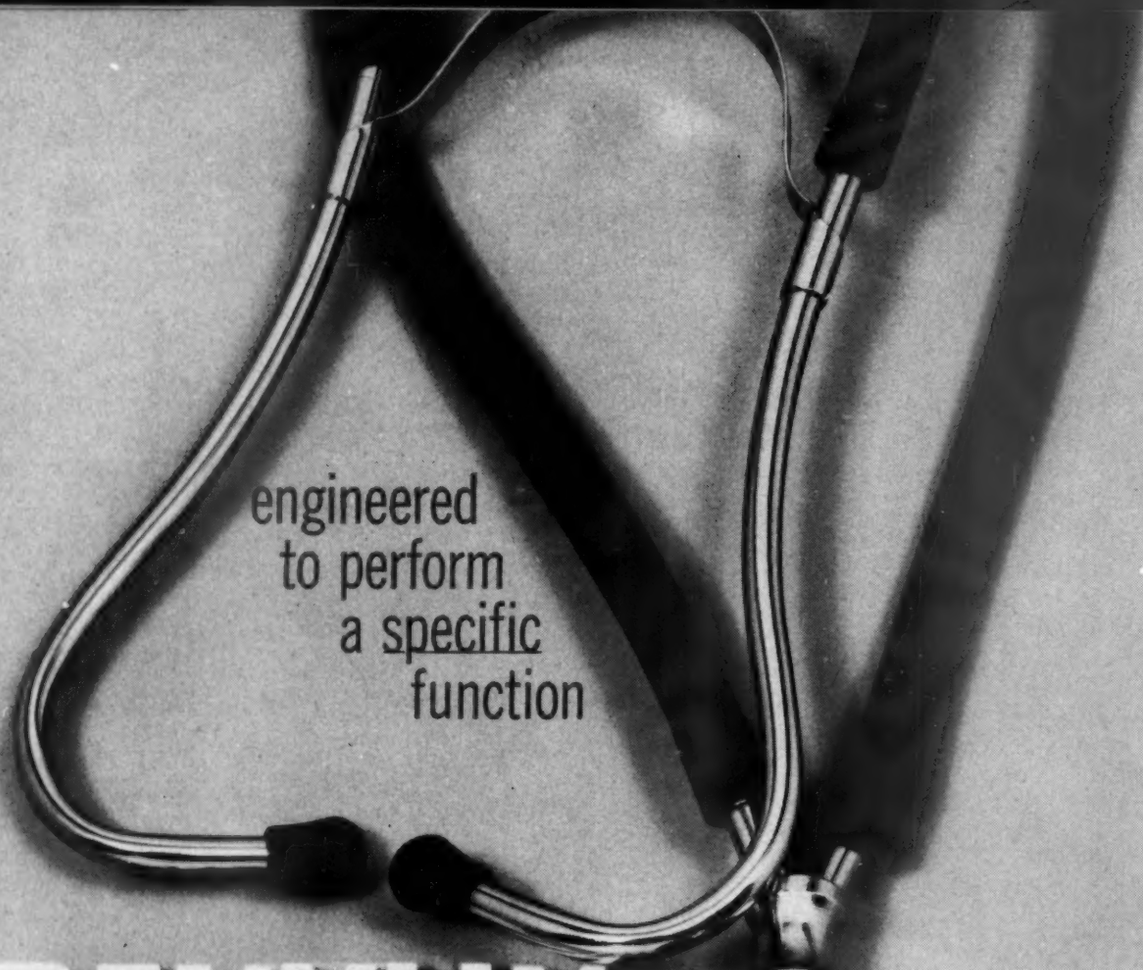
SUPPLIED: Tablets #2 (light orange), each containing 0.1 mg. Serpasil and 50 mg. Esidrix; bottles of 100. Tablets #1 (light orange), each containing 0.1 mg. Serpasil and 25 mg. Esidrix; bottles of 100.

SERPASIL® (reserpine CIBA)
ESIDRIX® (hydrochlorothiazide CIBA)

For complete information about Serpasil-Esidrix (including dosage, cautions, and side effects), see 1961 Physicians' Desk Reference or write CIBA, Summit, N.J. 2/2960MB

CIBA
SUMMIT·NEW JERSEY

Serpasil®-Esidrix®
(reserpine and hydrochlorothiazide CIBA)



engineered
to perform
a specific
function

BENYLIN EXPECTORANT

specifically designed to help control cough

Just as a medical instrument is engineered for maximum efficiency in performing its specific function, BENYLIN® EXPECTORANT is formulated to provide effective relief of cough associated with colds or allergy.

The outstanding antitussive action of BENYLIN EXPECTORANT is attributed to a combination of carefully selected therapeutic agents. Benadryl,® a potent antihistaminic-antispasmodic, reduces bronchial spasm, quiets the cough reflex, and lessens nasal stuffiness, sneezing, lacrimation, itching, and other allergic manifestations. Concurrent respiratory congestion is relieved by expectorant agents that efficiently break down tenacious mucosal secretions. In addition, a demulcent action soothes irritated throat membranes. 59961

BENYLIN EXPECTORANT is a pleasant-tasting, raspberry-flavored syrup...completely acceptable to patients of all ages.

supplied: BENYLIN EXPECTORANT is available in 16-ounce and 1-gallon bottles.

Each fluidounce contains: 80 mg. Benadryl Hydrochloride (diphenhydramine hydrochloride, Parke-Davis); 12 gr. ammonium chloride; 5 gr. sodium citrate; 2 gr. chloroform; 1/10 gr. menthol; and 5% alcohol. *Indications:* Relief of coughs due to colds, other symptoms associated with colds, and coughs of allergic origin. *Dosage:* Adults—1 to 2 teaspoonfuls every three to four hours. Children—½ to 1 teaspoonful every four hours. *Precautions:* Products containing Benadryl should be used cautiously with hypnotics or other sedatives; if atropine-like effects are undesirable; or if the patient engages in activities requiring alertness or rapid, accurate response (such as driving).

PARKE-DAVIS

PARKE, DAVIS & COMPANY, Detroit 32, Michigan

Schering

First total regimen in athlete's foot

ADVICIN is the first topical preparation to combine the proved *anhidrotic* and *antipruritic* benefits of an anticholinergic with widely accepted *fungicidal* and *keratolytic* agents... ADVICIN reduces local sweating, helps keep feet dry... helps relieve itching promptly... has a pleasant medicinal scent... may shorten the fungous-clearing time required with oral FULVICIN. *Supplied:* ADVICIN Powder—2 ounce can—for daytime use. ADVICIN Cream—50 gram tube—for nighttime use. For complete details, consult latest Schering literature available from your Schering Representative or Medical Services Department, Schering Corporation, Bloomfield, New Jersey.

S-874

NEW

advicin™

TOPICAL

first topical fungicide with sweat-inhibiting action*

2-way antifungal attack in a moisture-controlled, antifungal environment

ORAL

fulvicin®

brand of griseofulvin

first orally effective antifungal antibiotic for ringworm

*ADVICIN contains diphemanil methylsulfate (PRANTAL®) 2%, undecylenic acid 5%, and salicylic acid 3%.

"Doctor...

I'm so tired all the time"

In most cases where lethargy and fatigue are a problem—in menopause, senility, convalescence, oversedation, and mild depression, for example—the gentle stimulant action of Ritalin safely restores normal physical and mental activity. Summarizing the results of therapy with Ritalin in 89 patients who were either chronically ill, convalescing, depressed, or oversedated, Natenshon* states: "They were alert, fatigue disappeared, and they could go all day without tiring."

SUPPLIED. *Tablets,* 5 mg. (pale yellow), 10 mg. (light blue), 20 mg. (peach colored).

For complete information about Ritalin (including dosage, cautions, and side effects), see 1961 Physicians' Desk Reference or write CIBA, Summit, N. J.

RITALIN® hydrochloride (methylphenidate hydrochloride CIBA)

*Natenshon, A. L.: Dis. Nerv. System *17*:392 (Dec.) 1956.

she'll be active again on

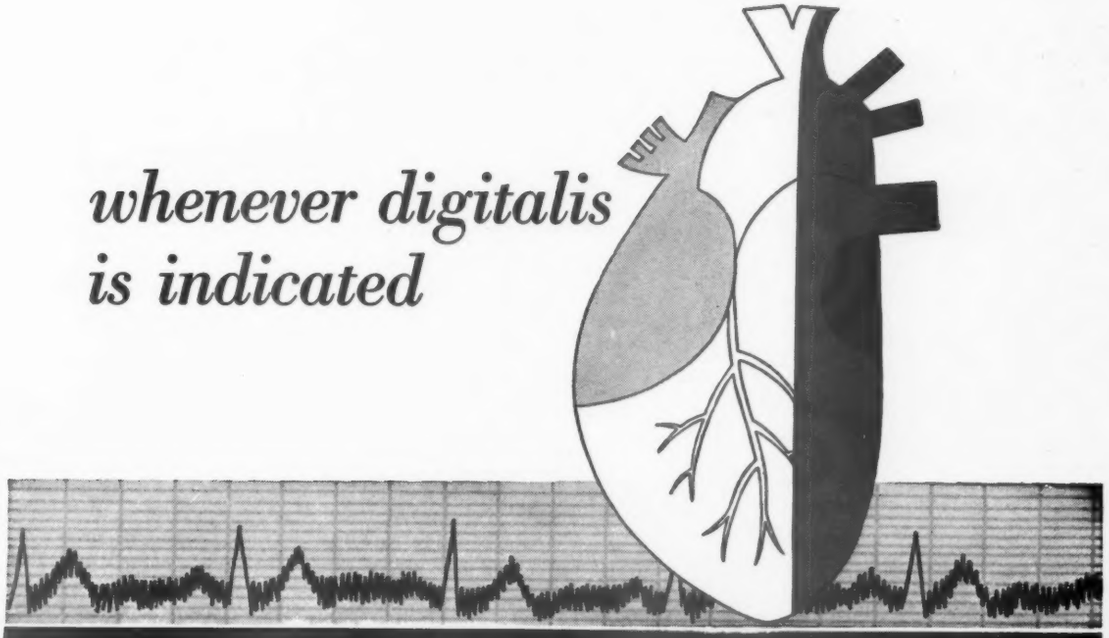
Ritalin®

gentle stimulant for lethargic patients

C I B A Summit, N. J.

3/297CM-B

whenever digitalis is indicated



'LANOXIN'® brand DIGOXIN

formerly known as Digoxin 'B. W. & Co.'

"If one digitalis agent were to be recommended for its adaptability to the many and varied clinical contingencies, we believe Digoxin would be the drug of choice."

Lown, B., and Levine, S. A.: Current Concepts in Digitalis Therapy, Boston, Little, Brown & Company, 1954, p. 23, par. 2.

'LANOXIN' TABLETS	'LANOXIN' INJECTION	'LANOXIN' ELIXIR PEDIATRIC
0.25 mg. scored (white)	0.5 mg. in 2 cc. (I.M. or I.V.)	0.05 mg. in 1 cc.
0.5 mg. scored (green)		

BURROUGHS WELLCOME & CO. (U.S.A.) INC., Tuckahoe, N.Y.

Male, Age 9; Dx: Poison Ivy Dermatitis.
Rx: Celestone, 1 tab. q.i.d. for two days, 1 tab. t.i.d. for two days, 1 tab. b.i.d. for one week, and 1 tab. daily for 7 days. Photograph shows the patient before treatment.

Allergic/inflammatory flare-up!

Results: Within 24 hours there was regression of intense inflammation and vesicles as well as a high degree of relief from itching. The patient had cleared completely at the end of ten days. Photograph after three days. (Photographs courtesy of M. Murray Nierman, M.D., Calumet City, Ill.)

Schering

A new achievement in corticosteroid activity: CELESTONE (betamethasone) has been called "perhaps the most important step ahead since the discovery of prednisone and prednisolone..."[1] and "unquestionably the most active adrenocortical steroid we have studied to date."[2] Pre-introductory clinical studies have established not only the high antiallergic/anti-inflammatory activity of CELESTONE but also its "low incidence of side effects... [and] absence of new toxic effects...."[3]

Three significant clinical advantages: In reporting results of a study of 154 dermatologic patients, treated up to 9 months, the investigators[1] cite as "three important clinical advantages of betamethasone [CELESTONE]: its almost uniform effectiveness at exceptionally low dosages, the striking absence of hormonal side effects in our series, and the ability of this corticosteroid to elicit a good therapeutic response in patients who had previously done poorly on other steroids."

Rapid remission with new Celestone

the first major advance in corticosteroid therapy in over 2½ years

Greater utility–ease of use: Gratifying results have been achieved with CELESTONE in a broad range of steroid-responsive disorders, from bronchial asthma and pollenosis to allergic dermatoses, inflammatory ocular diseases and rheumatoid arthritis. Rapid subsidence of allergic or inflammatory flare-up can usually be expected on average daily dosages of from 2 to 8 tablets. The single tablet strength (0.6 mg.) simplifies dosage schedules and facilitates proper dosage adjustment when patients are switched from other corticosteroids.

H-319

Safety-speed factor: Results with CELESTONE in 353 dermatologic patients[2] indicate that "its high degree of effectiveness and virtual absence of side effects in low dosages, which permit a simplified therapeutic regimen, make betamethasone [CELESTONE] an exceptionally useful corticosteroid in acute, short-term conditions."

For complete details, consult latest Schering literature available from your Schering Representative or the Medical Services Department, Schering Corporation, Bloomfield, New Jersey.

Bibliography: 1. Gant, J.Q., and Gould, A.H.: Betamethasone: A Clinical Study. Paper presented at First Conference on the Clinical Application of Betamethasone – A New Corticosteroid, New York City, May 8, 1961. 2. Nierman, M.M.: The Use of Betamethasone in Dermatology. *Ibid.* 3. Frank, L.: The Place of Betamethasone in Dermatologic Practice. *Ibid.*

NEW CELESTONE™

(betamethasone) **Tablets 0.6 mg.**

Put your low-back patient back on the payroll

Soma's prompt relief of pain and stiffness can get your low-back patients back to work in days instead of weeks

Soma is unique because it combines the properties of an effective muscle relaxant and an independent analgesic in *a single drug*. Unlike most other muscle relaxants, which can only relax muscle tension, Soma attacks both phases of the pain-spasm cycle at the same time.

Thus with Soma, you can break up both pain and spasm fast, effectively . . . help give your patient the two things he wants most: relief from pain and rapid return to full activity.

Soma is notably safe. Side effects are rare. Drowsiness may occur, but usually only with higher dosages. Soma is available in 350 mg. tablets. Usual dosage is 1 tablet q.i.d.

The muscle relaxant with an independent pain-relieving action

SOMA®

(carisoprodol, Wallace)

Wallace Laboratories, Cranbury, New Jersey

How you can help save your patients a month's pay

Kestler reports in J.A.M.A. (April 30, 1960) that conventionally treated low-back syndrome patients required an average of 41 days for full recovery (range: 3 to 90 days). The addition of Soma therapy in this comparative investigation reduced the average to 11.5 days (range: 2 to 21 days). With Soma, patients averaged full recovery 30 days sooner.

Notable Success with VISTARIL...

in prepartum tension and anxiety		allays anxiety without impairing ability to cooperate during labor and delivery[1]	reduces narcotic requirements and incidence of narcotic-induced respiratory depression, helps control emesis[1,4]
in the cardiac or the hypertensive patient		allays anxiety without adverse influence on blood pressure[2]	helps correct certain functional arrhythmias, does not increase gastric secretion[2]
in problem drinkers		allays anxiety—makes patient more manageable[3]	produces no significant depression of blood pressure, pulse rate, or respiration. No liver involvement reported
in preoperative tension and anxiety		allays anxiety without depression of vital functions[4]	reduces incidence of narcotic-induced respiratory depression and hypotension, relaxes skeletal muscle, smooths recovery and helps control emesis[4]
in pediatrics		allays tension in agitated, hyperkinetic patients	avoids danger of liver damage or other untoward reactions

References: **1.** Benson, C., and Benson, R. C.: Scientific Exhibit, Illinois Acad. Gen. Practice, Sept., 1960. **2.** Salmons, J. A.: Dis. Chest 38:105, 1960. **3.** Major, R. A.: GP 21:104, 1960. **4.** Grady, R. W., and Rich, A. L.: Scientific Exhibit, Am. Soc. Anesth., New York, Oct. 4-7, 1960.

for successful
tranquilization—

Vistaril

ORAL/HYDROXYZINE PAMOATE
PARENTERAL/HYDROXYZINE HYDROCHLORIDE

effectively allays anxiety

no reported incidence of liver damage, respiratory depression or addiction

exerts helpful antiemetic, antisecretory, antipruritic effects

Science for the world's well-being®

Pfizer

PFIZER LABORATORIES
Division, Chas. Pfizer & Co., Inc.
Brooklyn 6, New York

IN BRIEF

Vistaril is hydroxyzine pamoate. The hydrochloride salt of hydroxyzine is used in the parenteral solution.

Vistaril acts rapidly in the symptomatic treatment of a variety of neuroses and other emotional disturbances manifested by anxiety, apprehension or fear—whether occurring alone or complicating a physical illness. Used preoperatively and prepartum, Vistaril controls anxiety and fear, permits a substantial reduction in the amount of meperidine or other narcotic required for satisfactory analgesia, and helps prevent emesis. Vistaril's calming effect usually does not impair discrimination, and is accompanied by direct and secondary muscle relaxation. No toxicity has been reported with Vistaril, and it has a remarkable record of freedom from reactions.

INDICATIONS: Vistaril is clinically effective in anxiety and tension states, senility, anxiety associated with various disease states, alcoholism, pre- and postpartum and pre- and postoperative tension and emesis, certain functional arrhythmias, and pediatric behavior problems.

ADMINISTRATION AND DOSAGE: Dosage varies with the state and response of each patient, rather than with weight and should be individualized by the physician for optimum results. *Recommended oral dosage:* In anxiety and tension states, senility, alcoholism, pre- and postoperative and pre- and postpartum tension and emesis: up to 400 mg. daily in divided doses. In anxiety associated with asthma, neurodermatoses, menopausal syndrome, digestive disorders, functional or essential hypertension, tension headaches: 50 mg. q.i.d. initially—adjust according to response. In cardiac arrhythmias: initial—25 mg. q. 6 h. until arrhythmia disappears; maintenance or prophylactic—50-75 mg. daily in divided doses. In pediatric behavior problems under 6 years: 50 mg. daily in divided doses. Six and over: 50-100 mg. daily in divided doses. *Recommended parenteral dosage:* In preoperative, obstetrical, and more emergent situations in other indications: 25-100 mg. I.M. or I.V. q. 4 h., p.r.n. In cardiac arrhythmias: 50-100 mg. I.M. stat, and q. 4-6 h., p.r.n.; maintain with 25 mg. b.i.d. or t.i.d.

SIDE EFFECTS: Drowsiness may occur in some patients; if so, it is usually transitory, disappearing within a few days of continued therapy or upon reduction of dosage. Dryness of mouth may be encountered at higher doses.

PRECAUTIONS: The potentiating action of hydroxyzine should be taken into account when the drug is used in conjunction with central nervous system depressants. Do not exceed 1 cc. per minute I.V. Do not give over 100 mg. per dose I.V. Parenteral therapy is usually for 24-48 hours, except when, in the judgement of the physician, longer-term therapy by this route is desirable.

SUPPLIED: VISTARIL *Capsules* (hydroxyzine pamoate)—25, 50, and 100 mg. VISTARIL *Oral Suspension* (hydroxyzine pamoate)—25 mg. per 5 cc. teaspoonful. VISTARIL *Parenteral Solution* (hydroxyzine hydrochloride)—10 cc. vials, 25 mg. per cc.; 2 cc. ampules, 50 mg. per cc.

More detailed professional information available on request.

Three of these women have vaginitis (trichomonal, monilial or mixed). Only comprehensive therapy can reach all three.

For every 2 cases of vaginitis caused by Trichomonas vaginalis alone, there is usually 1 case caused by Candida (Monilia) albicans, Haemophilus vaginalis, or mixed infection involving several pathogens.[1-8]

You can reach all of these vaginitis patients with the *comprehensive* vaginal preparation effective against C. albicans, H. vaginalis and other bacterial pathogens, *in addition to* T. vaginalis.

1. POWDER *for weekly application in your office:* FUROXONE® (furazolidone) 0.1% and MICOFUR® (nifuroxime) 0.5%, in an acidic water-dispersible base. 15 Gm. plastic squeeze bottle. 2. SUPPOSITORIES *for continued home use:* first week 1 in the morning and 1 on retiring. After first week, 1 at night may suffice. Continue treatment during menses and throughout menstrual cycle and for several days thereafter. Contain MICOFUR 0.375% and FUROXONE 0.25% in a water-miscible base. Boxes of 12 or 24 suppositories with applicator.

TRICOFURON®

Improved

1. Coolidge, C. W.; Glisson, C. S., Jr., and Smith, A. A.: J. M. A. Georgia 48:167 (Apr.) 1959. 2. Ensey, J. E.: Am. J. Obst. & Gynec. 77:155 (Jan.) 1959. 3. Frech, H. C., and Lanier, L. R., Jr.: J. M. A. Georgia 47:498 (Oct.) 1958.

EATON LABORATORIES
Division of The Norwich Pharmacal Company
NORWICH, NEW YORK

if it is a muscleitis or bursitis it is Sigmagen® responsive

For six years in the vast muscle-itis and bursitis areas where analgesics fail to provide adequate relief, SIGMAGEN has offered greater certainty of clinical success.

SIGMAGEN provides a conservative, in-between level of therapy—far more capable than analgesics, yet not approaching high steroid dosage levels.

Your use of SIGMAGEN will swiftly allay the pain and quiet the inflammatory processes in mild rheumatoid arthritis, bursitis, myositis and fibrositis.

Meticorten® (prednisone)/ the classic steroid therapy	0.75 mg.
Acetylsalicylic acid/ for anti-inflammatory-analgesic action	325 mg.
Aluminum hydroxide/ buffering for better toleration	75 mg.
Ascorbic acid/ anti-stress supplementation	20 mg.

For complete details, consult latest Schering literature available from your Schering Representative or Medical Department, Schering Corporation, Bloomfield, New Jersey.

Bibliography: 1. Cohen, A., et al.: J.A.M.A. 165: 225, 1957. 2. Spies, T. D., et al.: J.A.M.A. 159: 645, 1955. 3 Moravec, C. L. and Moravec, M. E.: Clin. Med. 7:2322, 1960.

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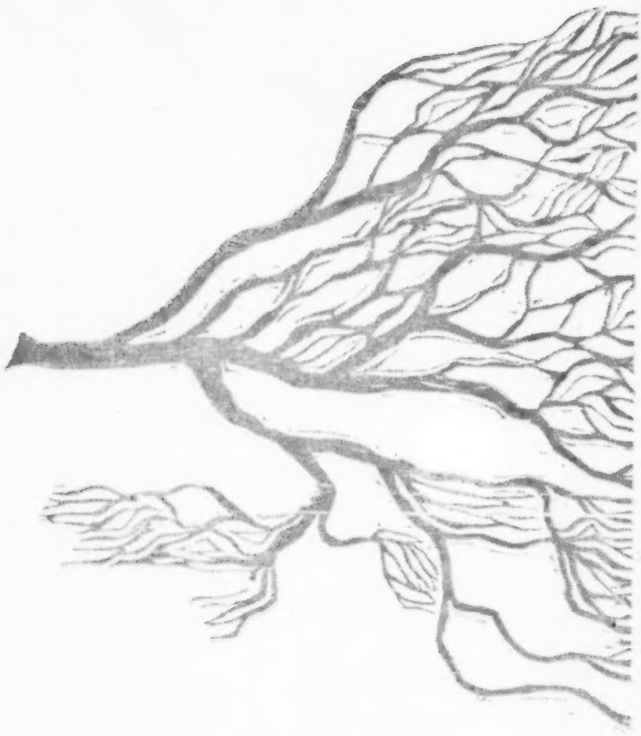
Sigmagen®

corticoid analgesic compound

Schering



in

HYPERTENSION

PHYSICIANS PRESCRIBE

DIURIL®

CHLOROTHIAZIDE

more often than any other diuretic

"Since the chlorothiazide compares well in effectiveness with other hypotensive drugs, it is our practice to initiate therapy with chlorothiazide alone in all patients with normal renal function. In the absence of signs indicating urgency in the reduction of pressure we find it advisable to continue such treatment for one or two months."

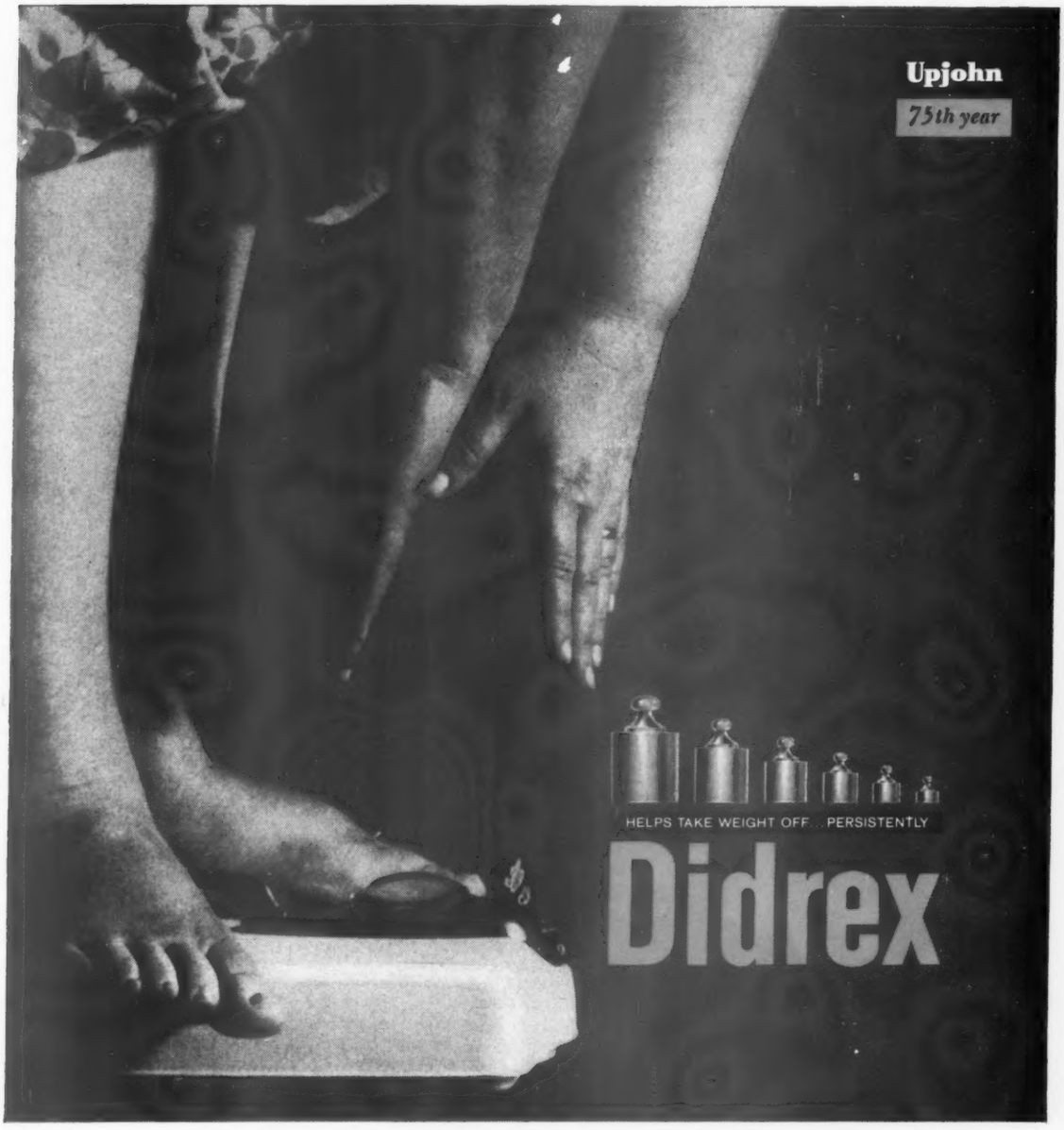
Conway, J., and Lauwers, P.: Circulation **21**:21, January, 1960.

Supplied: 250-mg. and 500-mg. scored tablets DIURIL chlorothiazide in bottles of 100 and 1000.

Before prescribing or administering DIURIL, the physician should consult the detailed information on use accompanying the package or available on request. DIURIL is a trademark of Merck & Co., INC.

MSD MERCK SHARP & DOHME
Division of Merck & Co., INC. West Point, Pa.

EFFECTIVE MANAGEMENT OF HYPERTENSION BEGINS WITH DIURIL



Didrex doesn't perform miracles... it just helps the obese patient do it herself.

The reason is simple: persistent, significant loss of weight, up to 30 weeks in reported cases, helps to preclude the "weight plateau" that so often discourages dieters after a few weeks. Thus, time and will become your allies in changing the patient's dietary habits built over months or years of weight accumulation. Didrex may be used in closely supervised diabetic, coronary insufficient, and hypertensive patients.

BRIEF BASIC INFORMATION

Description: Didrex is the Upjohn brand of benzphetamine hydrochloride [(+)-N-benzyl-N, α-dimethyl-phenethylamine hydrochloride]. A sympathomimetic compound with marked anorectic action and relatively little stimulating effect on the CNS or cardiovascular system.

Indications: Control of exogenous obesity.

Contraindications: None known to date. However, use with caution in moderate or severe hypertension, thyrotoxicosis, acute coronary disease, or cardiac decompensation.

Dosage: Initiate appetite control with ½ to 1 tablet (25 to 50 mg.) in mid-morning or mid-afternoon, according to the patient's eating habits for several days. Then "adjust" dosage to suit each patient's needs to a maximum of 3 tablets daily (150 mg.).

Side Effects: No effects on blood, urine, renal or hepatic functions have been noted. Minimal side effects have been observed occasionally: dry mouth, insomnia, nausea, palpitations and nervousness.

Supplied: 50 mg., benzphetamine hydrochloride, press-coated, scored tablets, in bottles of 100 and 500.

*Trademark—brand of benzphetamine hydrochloride, Upjohn.

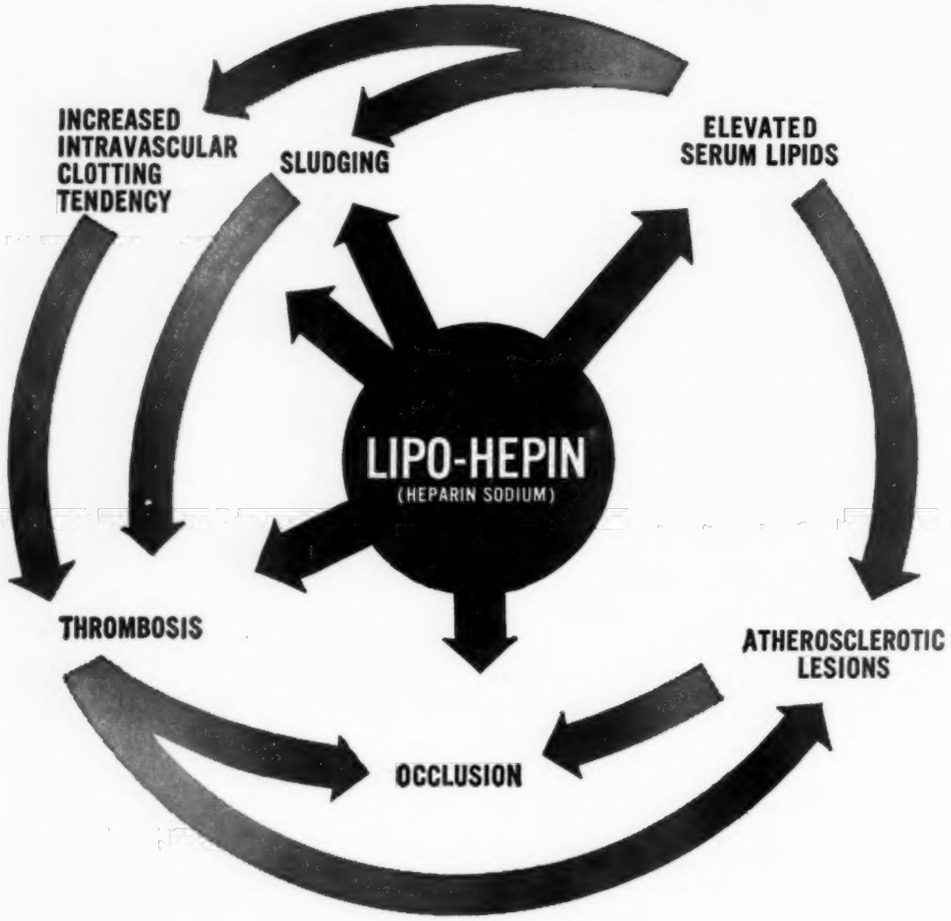
References: 1. Stough, A. R.: Weight loss without diet worry: use of benzphetamine hydrochloride (Didrex). Journal of the Oklahoma State Medical Association, 53:760-767 (November) 1960. 2. Oster, H., and Medlar, R.: A clinical pharmacologic study of benzphetamine (Didrex), a new appetite suppressant. Arizona Medicine, 17:398-404 (July) 1960. 3. Simkin, B., and Wallace, L.: A controlled clinical trial of benzphetamine (Didrex). Current Therapeutic Research, 2:33-38 (February) 1960.

Positive Antithrombotic Therapy

HEPARIN

is the only substance that protects against the organization and extension of thrombi by acting on both the clotting mechanism and on elevated lipid levels.

There is a growing body of evidence for inter-relationships among elevated serum lipids, atherosclerosis, hypercoagulability, and thrombosis.



LIPO-HEPIN

HEPARIN SODIUM INJECTION, AQUEOUS

Riker RIKER LABORATORIES, INC.
NORTHRIDGE, CALIFORNIA

Hostile Environment...

The fungus, protozoa and bacteria that commonly cause mild and severe leukorrhea require a vaginal pH of 5 to 12 for proliferation.

Trimagill creates a hostile environment! It produces a pH of 2.0 to 2.5—*the three principal infecting organisms cannot live in this acid range.*

Trimagill is well tolerated and has been proved effective in thousands of cases of leukorrhea, vaginitis, cervicitis, moniliasis and mixed infections. No untoward reactions that would require discontinuation of treatment were reported. At times denuded mucous membranes are so irritated that Trimagill may give a *temporary* burning sensation. This is usually short lived.

Trimagill does not foster resistant mutants or result in monilia overgrowth. Trimagill may be used during menstruation.

CONTENTS: Tartaric Acid, Citric Acid, Boric Acid, Dextrose, Potassium Alum, Potassium Bitartrate and Adhesives.

SUPPLIED: Powder: 5-oz. Plastic Insufflator Bottles; Vaginal Inserts: Boxes of 24.

NOTE: Consult package circular for information on dosage and instructions for use.

Write for descriptive literature.

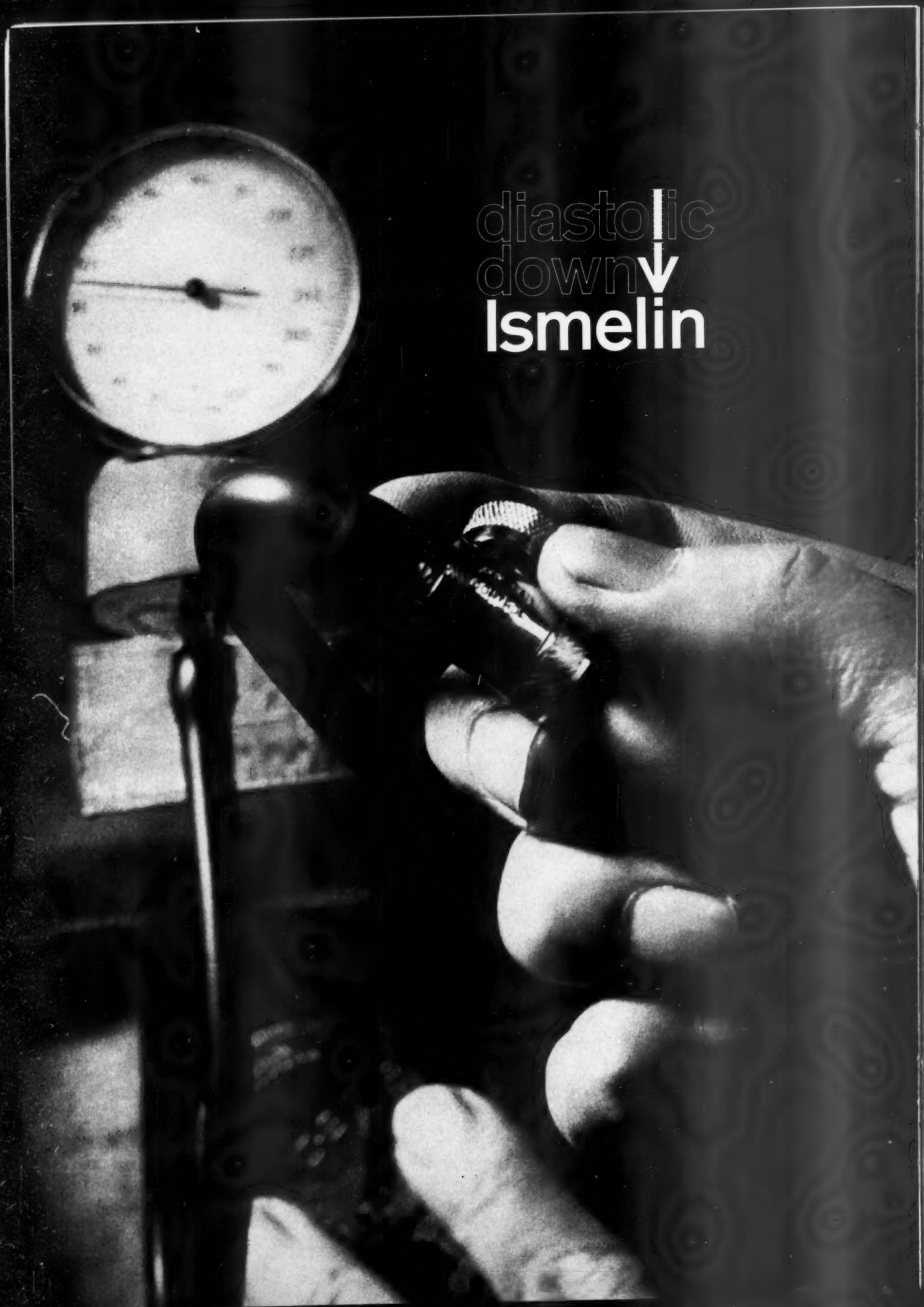
TRIMAGILL®*

POWDER • VAGINAL INSERTS

THE S. E. MASSENGILL COMPANY

Bristol, Tennessee • New York • Kansas City • San Francisco

*Patent applied for.


diastolic
down
Ismelin

"This new antihypertensive agent holds particular promise for those patients with the more severe degrees of diastolic blood pressure elevation."[1]

Elevated diastolic pressure of "crucial importance."[2] Increased peripheral resistance, as reflected by elevation of diastolic blood pressure, has been described as: "The single most important factor in the production of the type of arterial hypertension with which the physician is usually concerned..."[2]

Ismelin, through its unique action at the nerve-arteriole junction, dilates the arterioles, thereby reducing peripheral resistance. The result is often a marked decrease in diastolic pressure, as confirmed repeatedly by clinical observation.

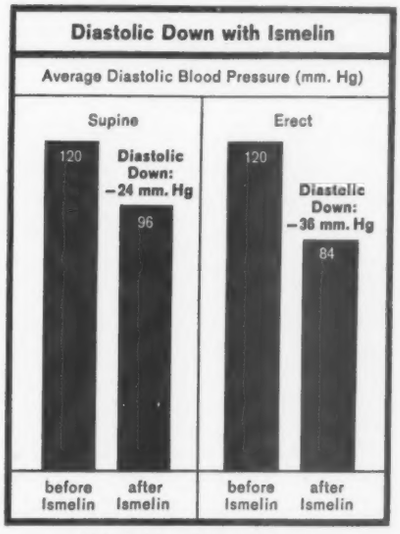
Ismelin lowers diastolic pressure after other treatments fail. Riven and Hall[3] studied Ismelin in 21 male hypertensive patients ranging in age from 30 to 69 years. Most patients were hospitalized initially, and most were treated with other antihypertensive drugs. Before treatment with Ismelin, all patients had diastolic pressures (supine and erect) of at least 110 mm. Hg "despite other antihypertensive therapy including ganglionic blocking agents."

All 21 patients responded to Ismelin* with "...a decrease in systolic and diastolic pressure in both supine and standing positions..."[3]

*When therapy with Ismelin began, mecamylamine was discontinued in 7 patients receiving it.

Advantages of Ismelin for your hypertensive patients

- Almost all forms of moderate to severe hypertension (including malignant hypertension and many cases of renal hypertension) can be managed with Ismelin — alone or in combination with other antihypertensive agents.
- Ismelin brings blood pressure down in many persons refractory to other antihypertensive agents.
- Ismelin lowers blood pressure in many patients who cannot be treated effectively with other potent agents because they do not tolerate the side effects.
- Patients need take Ismelin but once a day.



(Adapted from Riven and Hall[3])

- Most patients have been treated with Ismelin for prolonged periods without developing tolerance to it (although instances of tolerance have been reported).
- Smooth absorption of Ismelin results in predictable blood pressure responses.

For complete information about Ismelin (including dosage, cautions, and side effects), see current Physicians' Desk Reference or write CIBA, Summit, N. J.

Supplied: Tablets, 10 mg. (pale yellow, scored) and 25 mg. (white, scored)

References: 1. Brest, A. N. Novack, P., and Moyer, J. H.: To be published. **2.** Harrison, T.R., Adams, R.D., Bennett, I.L.Jr., Resnick, W. H., Thorn, G. W., and Wintrobe, M. M. (Editors): **Principles of Internal Medicine,** The Blakiston Division, McGraw-Hill Book Company, Inc., New York, 1958, p. 1321. **3.** Riven, S. S., and Hall, W.: South. M. J. 54:673 (June) 1961.

2/2986 MB

ISMELIN® sulfate (guanethidine sulfate CIBA)

diastolic down **Ismelin®**

CIBA Summit, New Jersey

BECAUSE POOR DIABETIC CONTROL INCREASES THE THREAT OF VASCULAR COMPLICATIONS IN DIABETES[1, 2]... DIABINESE FIRST FOR ADEQUATE AND CONTINUOUS ORAL CONTROL

Oral therapy with DIABINESE can help assure more adequate blood-sugar control in many maturity-onset diabetics, including certain patients now poorly controlled by diet alone, some patients on insulin, and many who escape control on previous oral therapy.

Diabinese and diet

In patients with maturity-onset diabetes whose blood sugar remains elevated despite weight and/or caloric control, DIABINESE is frequently effective in doses of 100 to 250 mg. a day. Further, unlike insulin, DIABINESE has not been reported to increase appetite, and residual capacity for endogenous beta cell activity is stimulated. Thus, DIABINESE combined with dietary regulation will often ensure more satisfactory control than "diet alone."

Diabinese and the insulin patient

DIABINESE has proved to be an effective replacement for insulin among maturity-onset patients needing 40 units or less per day. This application of DIABINESE is especially valuable in patients who should not be exposed to the hazards and inconvenience of self-administered injection—those with poor eyesight, the infirm and elderly, and the emotionally disturbed. Transfer from insulin to DIABINESE in proper dosage lessens the risk of hypoglycemia, and may enable certain patients to resume occupations where insulin shock is considered dangerous.

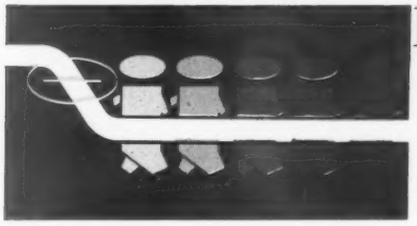
In selected patients in whom insulin requirements have become quite high, combined therapy with DIABINESE sometimes permits reduction of insulin dosage and helps to improve control.[3] Patients with insulin resistance may sometimes be similarly helped by replacement of part of the daily insulin dosage.[4]

Diabinese from the start

Continuous control in suitable candidates for sulfonylurea therapy is more likely to be achieved with DIABINESE. According to the A.M.A. Council on Drugs,[5] observations indicate that "on an equivalent dose and blood level basis, chlorpropamide has a somewhat greater therapeutic effect than has tolbutamide." This therapeutic superiority is reflected in the results of clinical observations like those of Fineberg,[6] who compared the effect of DIABINESE in 50 patients with the effect of tolbutamide in 35 patients. He concluded that "chlorpropamide produced satisfactory control of the diabetes in almost twice as great a percentage (76 versus 43 per cent) of patients than did tolbutamide, and excellent control in more than twice as great a percentage (74 versus 31 per cent)."

1. Johnsson, S.: Diabetes 9:1, 1960. 2. El Mahallawy, M. N., and Sabour, M. S.: J.A.M.A. 173:1783, 1960. 3. Editorial: Brit. M. J. 1:188, 1961. 4. Duncan, L. J. P., and Baird, J. D.: Pharmacol. Rev. 12:91, 1960. 5. A.M.A. Council on Drugs: New and Nonofficial Drugs, 1961, Philadelphia, Lippincott, 1961, p. 657. 6. Fineberg, S. K.: J. Am. Geriat. Soc. 8:441, 1960.

FOR MAXIMUM ASSURANCE OF CONTINUOUS BLOOD-SUGAR CONTROL



Diabinese®

BRAND OF CHLORPROPAMIDE

the oral antidiabetic most likely to succeed

economical once-a-day dosage

IN BRIEF

DIABINESE, a potent sulfonylurea, provides smooth, long-lasting control of blood sugar permitting economy and simplicity of low, once-a-day dosage. Moreover, DIABINESE often works where other agents have failed to give satisfactory control.

INDICATIONS: Uncomplicated diabetes mellitus of stable, mild or moderately severe nonketotic, maturity-onset type. Certain "brittle" patients may be helped to smoother control with reduced insulin requirements.

ADMINISTRATION AND DOSAGE: Familiarity with criteria for patient selection, continued close medical supervision, and observance by the patient of good dietary and hygienic habits are essential.

Like insulin, DIABINESE dosage must be regulated to individual patient requirements. Average maintenance dosage is 100-500 mg. daily. For most patients the recommended starting dose is 250 mg. given once daily. Geriatric patients should be started on 100-125 mg. daily. A priming dose is not necessary and should not be used; most patients should be maintained on 500 mg. or less daily. Maintenance dosage above 750 mg. should be avoided. Before initiating therapy, consult complete dosage information.

SIDE EFFECTS: In the main, side effects, e.g., hypoglycemia, gastrointestinal intolerance, and neurologic reactions, are related to dosage. They are not encountered frequently on presently recommended low dosage. There have been, however, occasional cases of jaundice and skin eruptions primarily due to drug sensitivity; other side effects which may be idiosyncratic are occasional diarrhea (sometimes sanguineous) and hematologic reactions. Since sensitivity reactions usually occur within the first six weeks of therapy, a time when the patient is under very close supervision, they may be readily detected. Should sensitivity reactions be detected, DIABINESE should be discontinued.

PRECAUTIONS AND CONTRAINDICATIONS: If hypoglycemia is encountered, the patient must be observed and treated continuously as necessary, usually 3-5 days, since DIABINESE is not significantly metabolized and is excreted slowly. DIABINESE as the sole agent is not indicated in juvenile diabetes mellitus and unstable or severely "brittle" diabetes mellitus of the adult type. Contraindicated in patients with hepatic dysfunction and in diabetes complicated by ketosis, acidosis, diabetic coma, fever, severe trauma, gangrene, Raynaud's disease, or severe impairment of renal or thyroid function. DIABINESE may prolong the activity of barbiturates. An effect like that of disulfiram has been noted when patients on DIABINESE drink alcoholic beverages.

SUPPLIED: As 100 mg. and 250 mg. scored chlorpropamide tablets.

More detailed professional information available on request.

Science for the world's well-being® Pfizer PFIZER LABORATORIES
Division, Chas. Pfizer & Co., Inc. New York 17, New York

One daily dose controls edema and hypertension around the clock

Every tablet delivers 24 hours' continuous thiazide action

ENDURON

Trademark

(Methyclothiazide, Abbott) Supplied in 2.5 and 5 mg. grooved tablets.

Enduron provides you the most sustained therapeutic effect of any thiazide available. Diuretic response is smooth and continuous. Even at end of the 24 hours, patients are still eliminating excess sodium well above control amounts.[1]

This unique duration permits convenient *once*-daily dosage—and without resulting gaps in the action. Easy for you to supervise, convenient for your patient—and fewer forgotten doses.

POTASSIUM CONTROL SIMPLIFIED

Potassium depletion is rare. Enduron's *single* daily dose causes but a *single* temporary peak of potassium loss.

Moreover, Enduron's effect on potassium has upper limits. Thus doubling the single dose from 5 to 10 mg. approximately doubles the output of sodium; yet under this same condition, potassium output increases little or not at all.[2]

Use Enduron to treat the edema of congestive heart failure, the nephrotic syndrome, hepatic cirrhosis, premenstrual tension, or steroid therapy. Enduron also demonstrates significant antihypertensive effect.[1,3] You may use it as a primary measure for mild to moderate hypertension, or as an adjunct in more severe cases.

1. Ford, R. V. Current Therap. Res., 2:422-430, Sept., 1960.
2. Fuchs, Morton, and Seller, Robert H., to be published.
3. Bryant, J. M., et al., Current Therap. Res., 3:1-4, Jan., 1961.

ABBOTT

109224

Where blood pressure climbs
—keep it under control with
Enduronyl

Announcing a once-a-day thiazide/rauwolfia antihypertensive

ENDURONYL

Trademark

(Methyclothiazide and Deserpidine, Abbott) (ENDURON™ and HARMONYL®)

Yes, just *once* daily is the dose for hypertension. Blood pressure starts down steadily: improvement usually is substantial within 10 days. Further reduction may continue over following weeks. At the same time, excess sodium and water are consistently eliminated.

Giving Enduronyl just once a day means fewer forgotten doses. A smoothly sustained therapeutic response, too.

On the other hand, single doses produce only one low, brief peak of potassium excretion. Hence one-a-day dosage means reduced chance of potassium depletion, since there's but one daily boost to the patient's potassium outgo.

WHAT IS ENDURONYL?

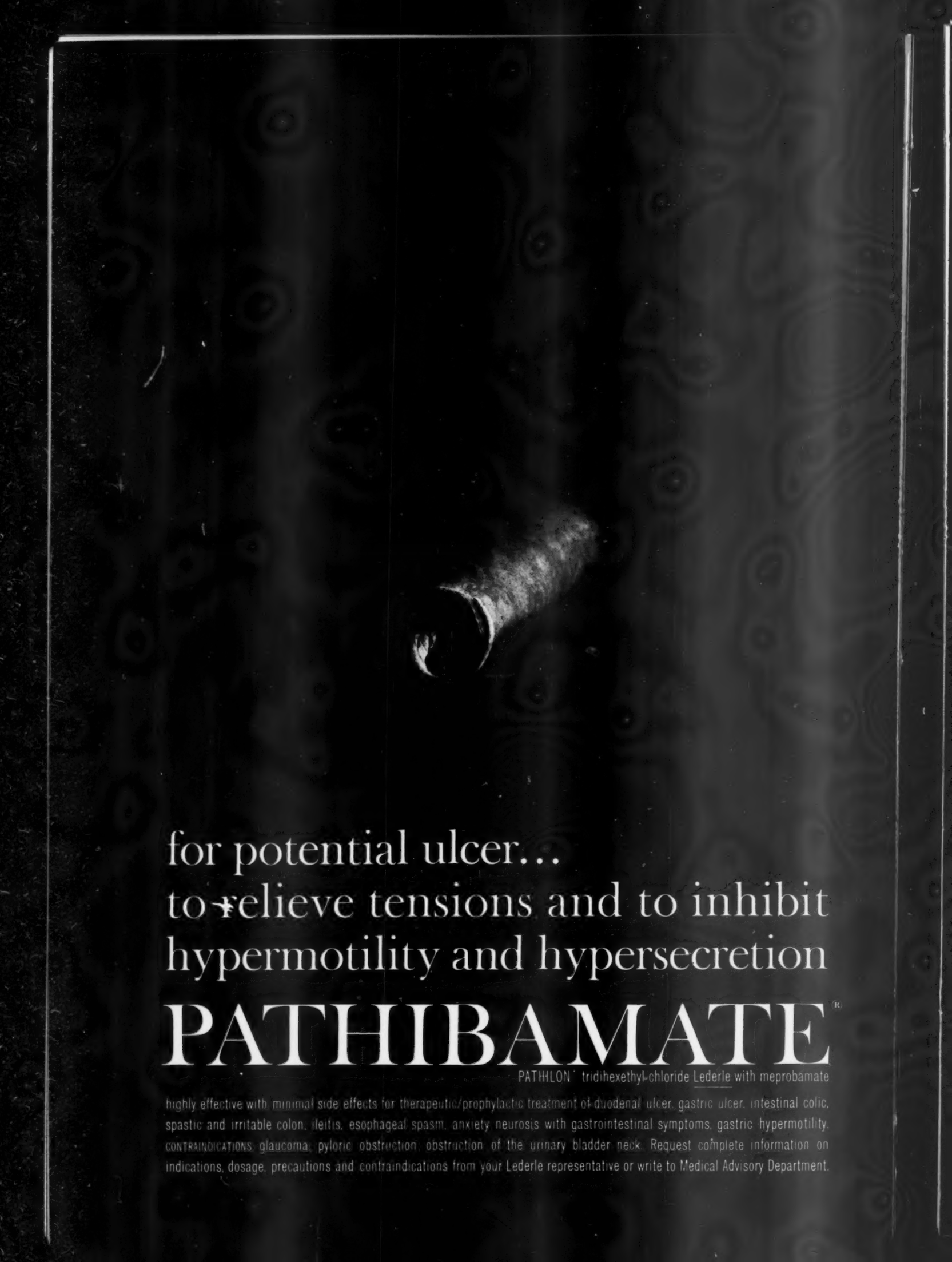
Enduronyl combines two well-established antihypertensive agents: (1) Enduron, Abbott's long-acting thiazide diuretic, and (2) Harmonyl, a rauwolfia derivative of low side effects. Together they provide greater hypotensive action than with either alone.

Enduronyl is supplied in two formulas, to cover a wide range of cases from mild to moderately severe hypertension. Would you like the literature? Write Abbott, North Chicago, Ill.

Supplied:
Enduronyl—(5 mg. Enduron, and 0.25 mg. Harmonyl)
Enduronyl Forte—(5 mg. Enduron, and 0.5 mg. Harmonyl)

ABBOTT

108225

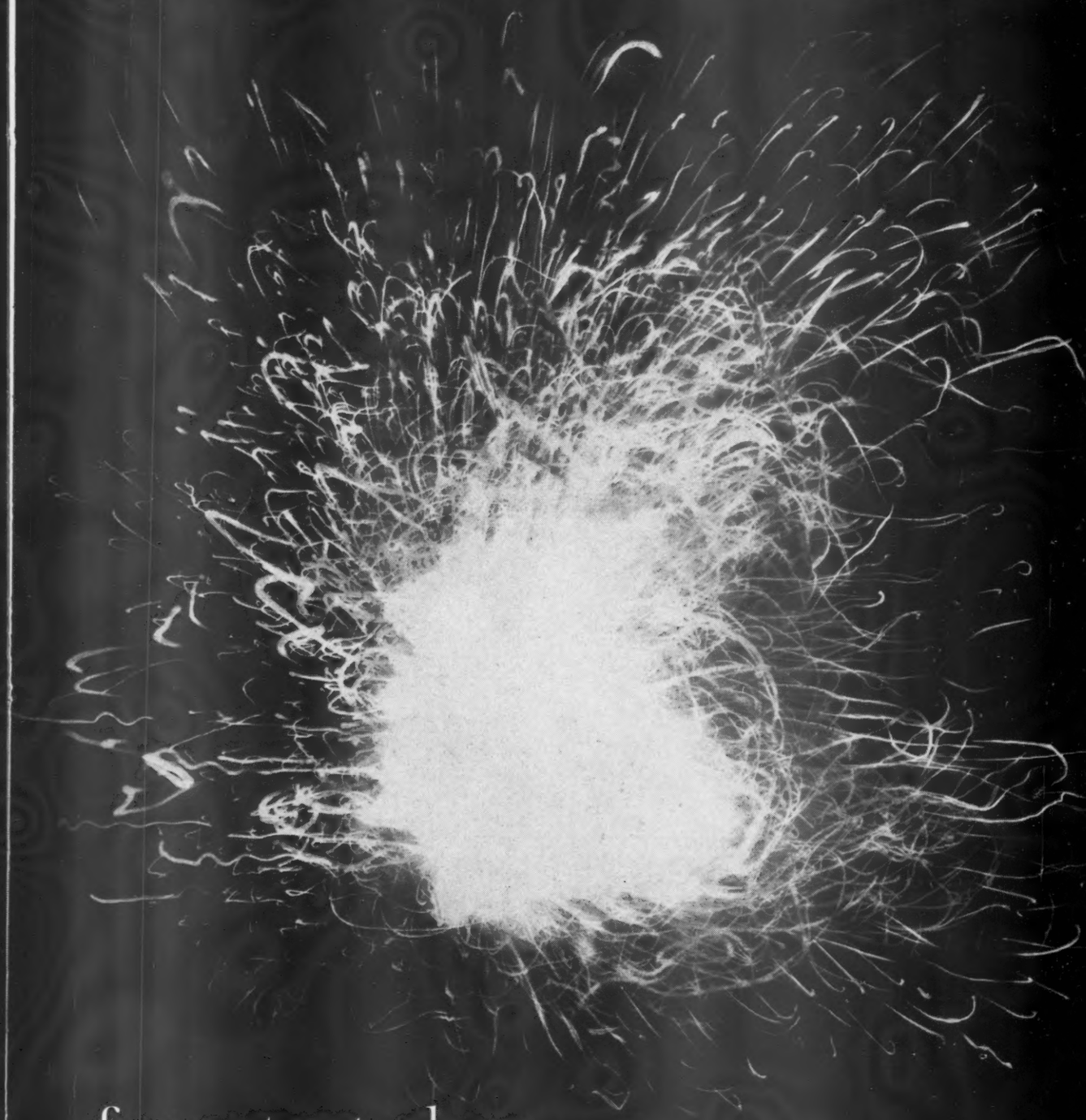


for potential ulcer...
to relieve tensions and to inhibit
hypermotility and hypersecretion

PATHIBAMATE®

PATHILON® tridihexethyl-chloride Lederle with meprobamate

highly effective with minimal side effects for therapeutic/prophylactic treatment of duodenal ulcer, gastric ulcer, intestinal colic, spastic and irritable colon, ileitis, esophageal spasm, anxiety neurosis with gastrointestinal symptoms, gastric hypermotility. CONTRAINDICATIONS: glaucoma, pyloric obstruction, obstruction of the urinary bladder neck. Request complete information on indications, dosage, precautions and contraindications from your Lederle representative or write to Medical Advisory Department.



for patent ulcer...
to relieve tensions and to inhibit
hypermotility and hypersecretion

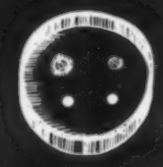
PATHIBAMATE

PATHIBAMATE-400 (full meprobamate effect)—1 tablet t.i.d. at mealtime, and 2 tablets at bedtime · PATHIBAMATE-200 (limited meprobamate effect)—1 or 2 tablets t.i.d. at mealtime, and 2 tablets at bedtime · Adjust to patient response. Each Pathibamate-200 tablet contains: PATHILON, 25 mg.; meprobamate, 200 mg. Pathibamate-400 tablets contain 400 mg. meprobamate.

Lederle LEDERLE LABORATORIES, A Division of AMERICAN CYANAMID COMPANY, Pearl River, New York

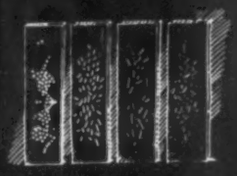
new Azo-Mandelamine

in common lower urinary infections



controls urinary infection without producing resistant mutants

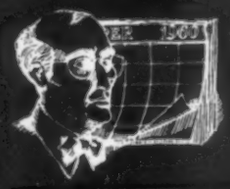
relieves urinary pain in 30 minutes



effective against most urinary pathogens

active only in the urinary tract

sensitization and other systemic reactions do not develop

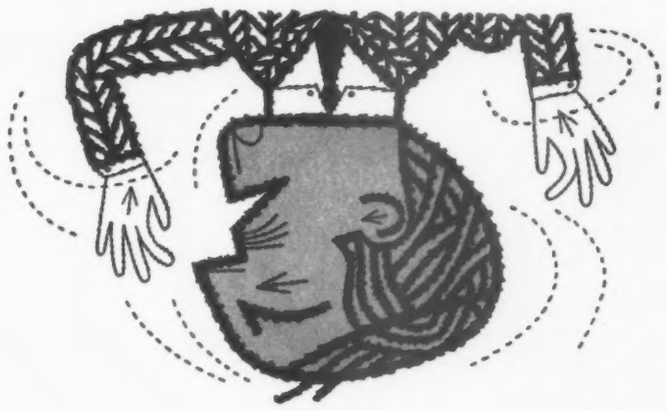


well tolerated

WARNER CHILCOTT

Composition: Each Azo-Mandelamine tablet contains 50 mg. phenylazodiamino-pyridine HCl (Pyridium®) and 500 mg. methenamine mandelate (Mandelamine®). **Average Adult Dosage:** Two Azo-Mandelamine tablets four times a day. **Precautions:** Azo-Mandelamine is contraindicated in patients with renal insufficiency and/or severe hepatitis. An occasional patient may experience gastrointestinal disturbance. Full dosage information, available on request, should be consulted before [illegible] therapy.

WARNER-CHILCOTT PRODUCTS ARE MARKETED BY [illegible] PRODUCTS [illegible]



vertigo is reversible

Antivert stops vertigo

moderate to complete relief of symptoms in 9 out of 10 patients[1]

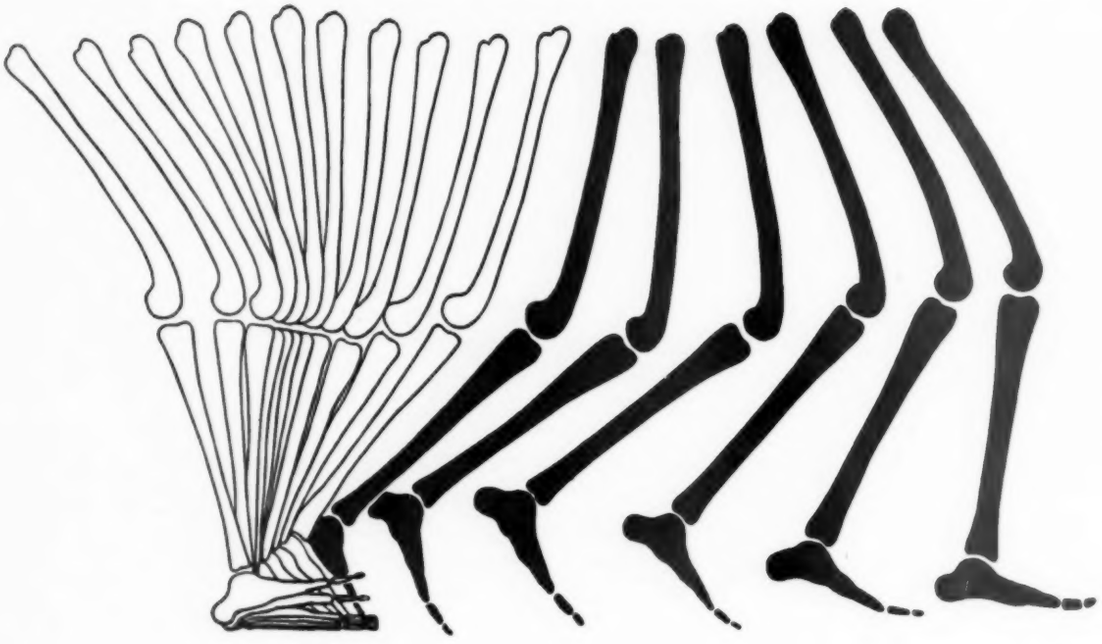
Prescribe one ANTIVERT tablet (or 1-2 teaspoonfuls ANTIVERT syrup) 3 times daily, before each meal, for prompt relief of vertigo, Meniere's syndrome and allied disorders. Side effects are short-lived, usually only harmless flushing and tingling associated with vasodilation. As with all vasodilators, ANTIVERT is contraindicated in severe hypotension and hemorrhage.

Supplied: Small blue-and-white scored tablets (meclizine HCl 12.5 mg. and nicotinic acid 50 mg.) in bottles of 100. Syrup (each 5 cc. teaspoonful contains meclizine HCl 6.25 mg. and nicotinic acid 25 mg.) in pint bottles. Prescription only. Bibliography available on request.

Reference: 1. Scal, J. C.: Eye Ear Nose & Throat Month. 38:738 (Sept.) 1959.

And for your aging patients—
NEOBON® Capsules
five-factor geriatric supplement

New York 17, N.Y.
Division, Chas. Pfizer & Co., Inc.
Science for the World's Well-Being®



Depo-Medrol was administered intra-articularly to 118 patients (250 injections) for disorders including rheumatoid arthritis, osteoarthritis, epicondylitis, and tendinitis.

Relief of pain and swelling was marked or complete in 104 of the 118 (88.1%); duration of response to a single injection was more than three weeks in 89 patients (75.4%) and more than six weeks in 39 of these.[1] "Post-injection flare-up was practically non-existent."[1]

relief within hours... lasting for weeks

Depo-Medrol* intra-articularly

Indications and dosages

Intra-articular, intrabursal and intratendinous injections of Depo-Medrol are useful for sustained anti-inflammatory effect and symptomatic relief in rheumatoid arthritis, osteoarthritis, bursitis, tendinitis, epicondylitis and other rheumatic disorders.

Intra-articular dosage depends on the size of the joint and the severity of the condition. Injections may be repeated, if necessary, at intervals of one to five weeks. A suggested dosage guide: Large joint, 20 to 80 mg.; medium joint, 10 to 40 mg.; small joint, 4 to 10 mg.

For administration directly into bursae, dosage may be 4 to 30 mg. (repeat injections are usually not needed).

For injection into the tendon sheath, 4 to 30 mg. is a usual range (in recurrent or chronic conditions, repeat injections may be needed).

Precautions

Depo-Medrol for local effect is contraindicated in the presence of acute infectious conditions. Infrequently, atrophic changes in the dermis may form shallow depressions in the skin at the injection site, but these usually disappear in a few months.

Depo-Medrol 40 mg. per cc.
Each cc. contains:

Medrol (methylprednisolone) acetate	40 mg.
Polyethylene glycol 4000	29 mg.
Sodium chloride	8.7 mg.
Myristyl-gamma-picolinium chloride	0.19 mg.
Water for injection	q.s.

Supplied: 1 cc. and 5 cc. vials

20 mg. per cc.
Each cc. contains:

Medrol (methylprednisolone) acetate	20 mg.
Polyethylene glycol 4000	29.6 mg.
Sodium chloride	8.9 mg.
Myristyl-gamma-picolinium chloride	0.19 mg.
Water for injection	q.s.

Supplied: 5 cc. vials

1. Norcross, B. M., and Winter, J. A.: Methylprednisolone acetate: a single preparation suitable for both intra-articular and systemic use, New York J. Med. *61*:552 (Feb. 15) 1961.

*Trademark, Reg. U. S. Pat. Off. methylprednisolone acetate, Upjohn

The Upjohn Company, Kalamazoo, Michigan

Upjohn

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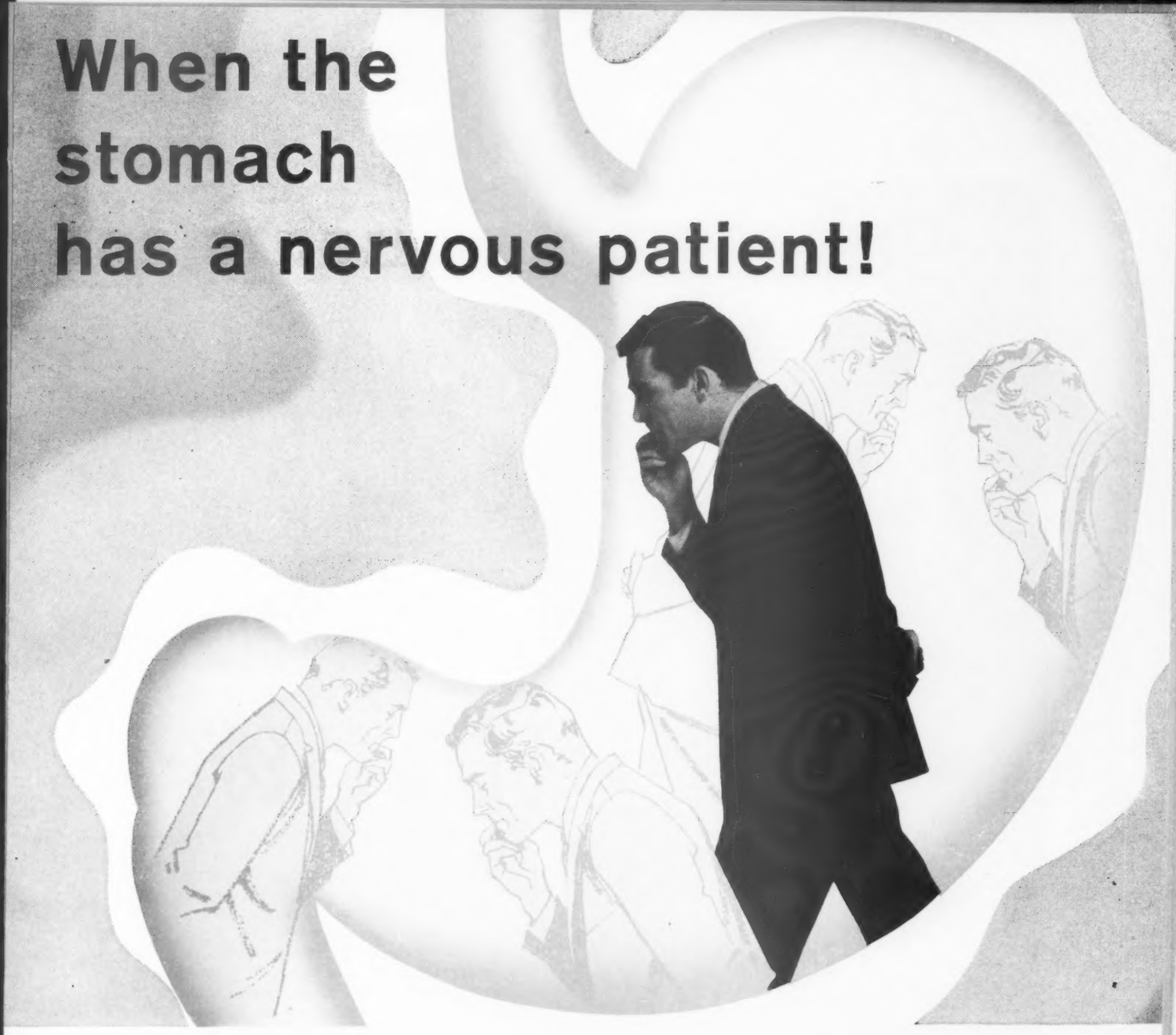
When the stomach has a nervous patient!



BUTIBEL®

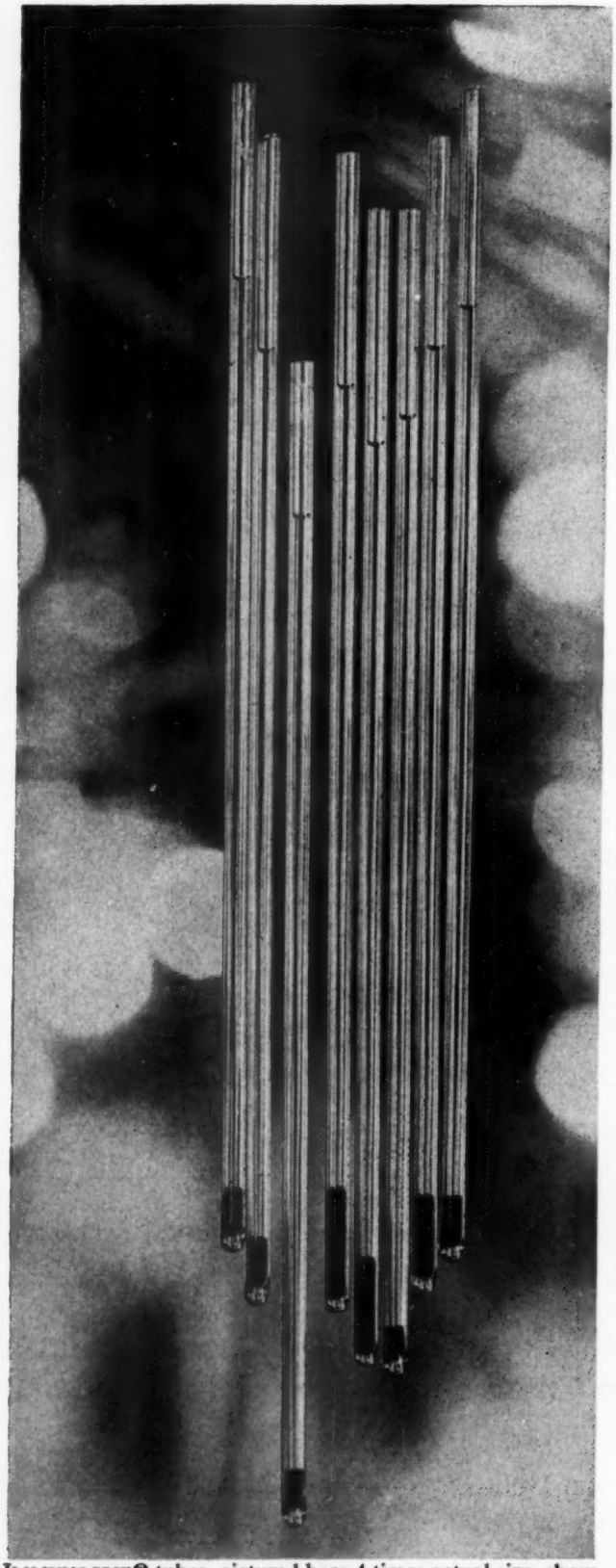
antispasmodic/sedative

relaxes the tense patient and his jittery stomach...without the sedative "build-up" many patients experience with phenobarbital preparations.

BUTIBEL combines the "time-matched" components—BUTISOL SODIUM® butabarbital sodium 15 mg. and extract of belladonna 15 mg.—each having approximately 5 hours' duration of effect. Thus, with Butibel there is no overlapping sedation, no antispasmodic gap—t.i.d. dosage keeps the patient comfortable without sluggishness.

Available as: BUTIBEL Tablets • Elixir • Prestabs® Butibel R-A (Repeat Action Tablets)

McNEIL McNEIL LABORATORIES, INC., Fort Washington, Pa.



IMMUNOCRIT® tubes, pictured here 4 times actual size, show results of centrifuging patients' serums with BETA-L TEST antiserum. Longest columns of precipitate indicate high beta-lipoprotein levels.

NEW...

A SPECIFIC SIMPLE INEXPENSIVE TEST FOR ESTIMATING SERUM BETA-LIPOPROTEIN LEVELS

BETA-L TEST™

The growing evidence implicating beta-lipoprotein as one of the agents responsible for atherosclerosis has emphasized the need for a test procedure which is reliable, yet simple enough to be suited to clinical purposes.[1] Hyland BETA-L TEST answers this need. BETA-L TEST results show a close correlation with complex quantitative methods now in use, yet BETA-L TEST can be performed in ten minutes on one drop of serum and employs a minimum of laboratory equipment and technical skill.[2]

The resulting economy and simplicity provide you with a reliable routine screening test and make frequent serial determinations of beta-lipoprotein practical for the first time.

When ordering your next beta-lipoprotein determinations, specify Hyland BETA-L TEST. Materials for BETA-L TEST are now available to your laboratory in 60-test kits.

1. Wood, F. C., Gurin, S., and Kuo, P. T.: Medical Correlation Clinic on Atherosclerosis and Coronary Artery Disease, Am. Pract.–Dig. Treat. *12:* 235 (April) 1961.

2. Heiskell, C. L., Fisk, R. T., Florsheim, W. H., Yachi, A., Goodman, J. R., and Carpenter, C. M.: A Simple Method for Quantitation of Serum Beta-Lipoproteins by Means of the Immunocrit, Amer. J. Clin. Path. *35:* 222 (March) 1961.

Hyland

HYLAND LABORATORIES

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reducing the problems of reducing

Preludin®

Tablets and Endurets®
prolonged-action tablets

brand of phenmetrazine HCl

**an oxazine...
not an amphetamine**

Unsurpassed Effectiveness
In all controlled clinical studies, Preludin has produced impressively greater weight loss than placebo tablets regardless of the degree of enforcement of dietary restriction.

Exceptionally High Tolerance
Reports are numerous of successful use of Preludin in cases intolerant of other anorexiants.

Flexibility of Dosage
Available as scored tablets of 25 mg. for b.i.d. or t.i.d. administration and also as Endurets®, 75 mg., for once daily administration.

Precautions and Contraindications
Although there have been no reports of significant toxic reactions to Preludin, on theoretical grounds it should not be given to patients with severe hypertension, thyrotoxicosis or acute coronary disease.

Preludin may be used with caution in cases of moderate hypertension and cardiac decompensation.

Preludin®, brand of phenmetrazine hydrochloride. Under license from C. H. Boehringer Sohn, Ingelheim.

Geigy Pharmaceuticals
Division of Geigy Chemical Corporation
Ardsley, New York

Geigy

PR 527-61

but it needn't trigger a respiratory crisis

You can't isolate the patient with chronic respiratory disease from all potential irritants. That is why Choledyl prophylaxis is essential. Taken regularly – daily – Choledyl helps prevent severe respiratory flare-ups by affording continuous relief from debilitating bronchospasm. Throughout long-term use, Choledyl is uniformly effective. And even in older patients, gastric upset and other unwanted effects are rare.

Dosage: Adults – 1 tablet q.i.d. *Supplied:* 200 mg. tablets (yellow), bottles of 100. *Precautions:* Side effects have been minimal but may include CNS stimulation or, rarely, palpitation. Full dosage information, available on request, should be consulted before initiating therapy.

TO AVOID THE CRISIS IN CHRONIC BRONCHITIS, CHRONIC ASTHMA, EMPHYSEMA

CHOLEDYL®

THE CHOLINE SALT OF THEOPHYLLINE

brand of oxtriphylline

WARNER CHILCOTT

MORRIS PLAINS, N.J.

GP 13

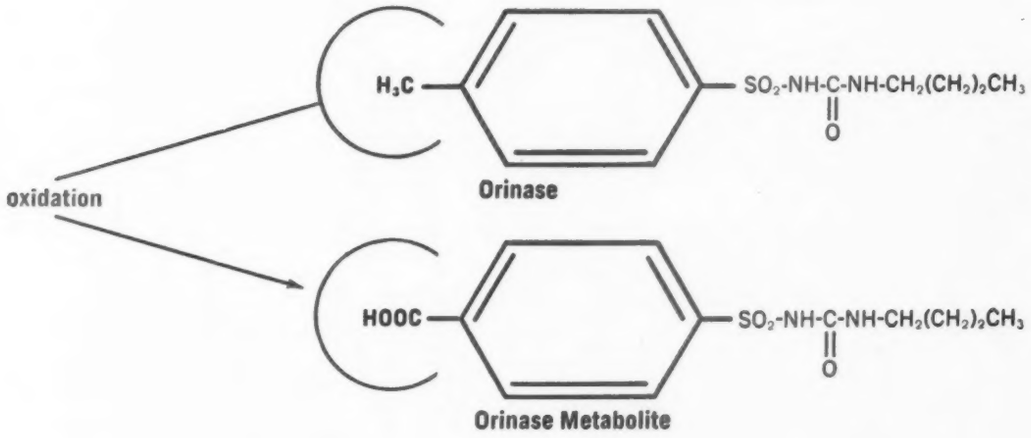
makers of Tedral Gelusil Proloid Peritrate Mandelamine

Why is the methyl "governor" in Orinase so important?

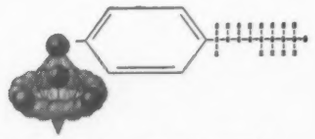
One of the most significant advantages of Orinase therapy is the rarity of associated hypoglycemic reactions.

This widely-reported clinical benefit is a function of the exclusive Orinase methyl "governor." Lending itself to ready oxidation (principally, it is thought, a hepatic process), the methyl group ensures prompt metabolic inactivation of the Orinase molecule. What actually happens is that a rapidly- and continuously-excreted carboxy-metabolite is produced that has no hypoglycemic activity at the existing levels.

As a result of the oxidation of its methyl group, Orinase shows a decline in activity soon after it reaches its effective peak in the plasma. Maintenance dosage serves to reduce blood sugar levels to normal, but rarely below that point, and there is no reported problem of accumulation.



Orinase*



An exclusive methyl "governor" minimizes hypoglycemia

Indications and effects: The clinical indication for Orinase is stable diabetes mellitus. Its use brings about the lowering of blood sugar; glycosuria diminishes, and such symptoms as pruritus, polyuria, and polyphagia disappear.

Dosage: There is no fixed regimen for initiating Orinase therapy. A simple and effective method is as follows: *first day*—6 tablets; *second day*—4 tablets; *third day*—2 tablets. The daily dose is then adjusted—raised, lowered or maintained at the two-tablet level, whichever is necessary to maintain optimum control.

Patients receiving insulin (less than 20 units)—discontinue insulin and institute Orinase; (20 to 40 units)—initiate Orinase with a concurrent 30 to 50% reduction in insulin dose with a further careful reduction as response to Orinase is observed; (more than 40 units)—reduce insulin by 20% and initiate Orinase with a further careful reduction in insulin dosage as response to Orinase is observed. In candidates for combined Orinase-insulin therapy, an individualized schedule is usually obtainable during a trial course of two or more weeks.

Contraindications and side effects: Orinase is contraindicated in patients having juvenile or growth-onset, unstable or brittle types of diabetes mellitus; history of diabetic coma, fever, severe trauma or gangrene.

Side effects are mild, transient and limited to approximately 3% of patients. Hypoglycemia and toxic reactions are extremely rare. Hypoglycemia is most likely to occur during the period of transition from insulin to Orinase. Other untoward reactions to Orinase are usually not of a serious nature and consist principally of gastrointestinal disturbances, headache, and variable allergic skin manifestations. The gastrointestinal disturbances (nausea, epigastric fullness, heartburn) and headache appear to be related to the size of the dose, and they frequently disappear when dosage is reduced to maintenance levels or the total daily dose is administered in divided portions after meals. The allergic skin manifestations (pruritus, erythema, and urticarial, morbilliform, or maculopapular eruptions) are transient reactions, which frequently disappear with continued drug administration. However, if the skin reactions persist, Orinase should be discontinued.

Clinical toxicity: Orinase appears to be remarkably free from gross clinical toxicity on the basis of experience accumulated during more than four years of clinical use. Crystalluria or other untoward effects on renal function have not been observed. Long-term studies of hepatic function in humans and experience in over 650,000 diabetics have shown Orinase to be remarkably free of hepatic toxicity. There has been reported only one case of cholestatic jaundice related to Orinase administration, which occurred in a patient with pre-existing liver disease and which rapidly reversed upon discontinuance of the drug.

Each tablet contains:
Tolbutamide ..... 0.5 Gm.

Supplied: In bottles of 50.

*Trademark, Reg. U.S. Pat. Off.—tolbutamide, Upjohn

June, 1961

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The Upjohn Company, Kalamazoo, Michigan

Upjohn

IRON: often a minus in moms and minors...

LIVITAMIN®

. . . the hematinic with built-in nutritional support

Many growing children and most women of menstrual age deplete their iron reserves and slide into iron-deficiency anemia.

Livitamin changes the minus to a plus because it restores depleted iron reserves and also provides integrated nutritional support.

Iron in Livitamin is well absorbed, with minimum gastric upset and constipation. And with Livitamin there is no worry about teeth stain . . . or taste acceptance.

WRITE FOR LITERATURE
AND DOSAGE INFORMATION.

FORMULA: Each fluidounce contains:

Iron, peptonized (equiv. in elemental iron to 71 mg.)	420 mg.
Manganese citrate, soluble, N.F.	158 mg.
Thiamine hydrochloride	10 mg.
Riboflavin	10 mg.
Cobalamin	20 mcg.
Nicotinamide	50 mg.
Pyridoxine hydrochloride	1 mg.
Pantothenic acid	5 mg.
Liver fraction 1	1 Gm.
Rice bran extract, U.S.P. XIV	1 Gm.
Inositol	30 mg.
Choline	60 mg.

SUPPLIED: *Liquid:* 8 oz. bottles, pints, gallons; *Capsules:* Bottles of 100, 500, 1000. Also available as LIVITAMIN with INTRINSIC FACTOR: bottles of 100 capsules.

THE S. E. MASSENGILL COMPANY

Bristol, Tennessee • New York • Kansas City • San Francisco

effective treatment for surgical infections

CHLOROMYCETIN®

(chloramphenicol, Parke-Davis)

Wound infection—a common postoperative complication—can very often be traced to staphylococcal invasion.[1-3] In such cases, CHLOROMYCETIN may well be an agent of choice, since "...the very great majority of the so-called resistant staphylococci are susceptible to its action."[4]

Contributing significantly to this preference is the fact that staphylococcal resistance to CHLOROMYCETIN remains surprisingly infrequent, despite widespread use of the drug.[5,6] For example, even though consumption of CHLOROMYCETIN at one hospital increased markedly since 1955, there was little change in the susceptibility of staphylococci to the drug.[6]

Characteristically broad in its range of antibacterial action, CHLOROMYCETIN has also proved valuable in surgical infections caused by other pathogens—both gram-positive *and* gram-negative.[7-8]

CHLOROMYCETIN (chloramphenicol, Parke-Davis) is available in various forms, including Kapseals® of 250 mg., in bottles of 16 and 100.

See package insert for details of administration and dosage.

Warning: Serious and even fatal blood dyscrasias (aplastic anemia, hypoplastic anemia, thrombocytopenia, granulocytopenia) are known to occur after the administration of chloramphenicol. Blood dyscrasias have occurred after both short-term and prolonged therapy with this drug. Bearing in mind the possibility that such reactions may occur, chloramphenicol should be used only for serious infections caused by organisms which are susceptible to its antibacterial effects. Chloramphenicol should not be used when other less potentially dangerous agents will be effective, or in the treatment of trivial infections such as colds, influenza, or viral infections of the throat, or as a prophylactic agent.

Precautions: It is essential that adequate blood studies be made during treatment with the drug. While blood studies may detect early peripheral blood changes, such as leukopenia or granulocytopenia, before they become irreversible, such studies cannot be relied upon to detect bone marrow depression prior to development of aplastic anemia.

References: (1) Pulaski, E. J., & Taylor, L. W.: *California Med.* 92:35, 1960. (2) Finland, M.: DM: Disease-a-Month, Sept., 1960, p. 3. (3) Monsour, V.; Bernard, H. R., & Cole, W. R.: *Missouri Med.* 57:1006, 1960. (4) Welch, H., in Welch, H., & Finland, M.: Antibiotic Therapy for Staphylococcal Diseases, New York, Medical Encyclopedia, Inc., 1959, p. 14. (5) Bauer, A. W.; Perry, D. M., & Kirby, W. M. M.: *J.A.M.A.* 173:475, 1960. (6) Petersdorf, R. G., *et al.*: *Arch. Int. Med.* 105:398, 1960. (7) Goodier, T. E. W., & Parry, W. R.: *Lancet* 1:356, 1959. (8) Lind, H. E.: *Am. J. Proctol.* 11:392, 1960.

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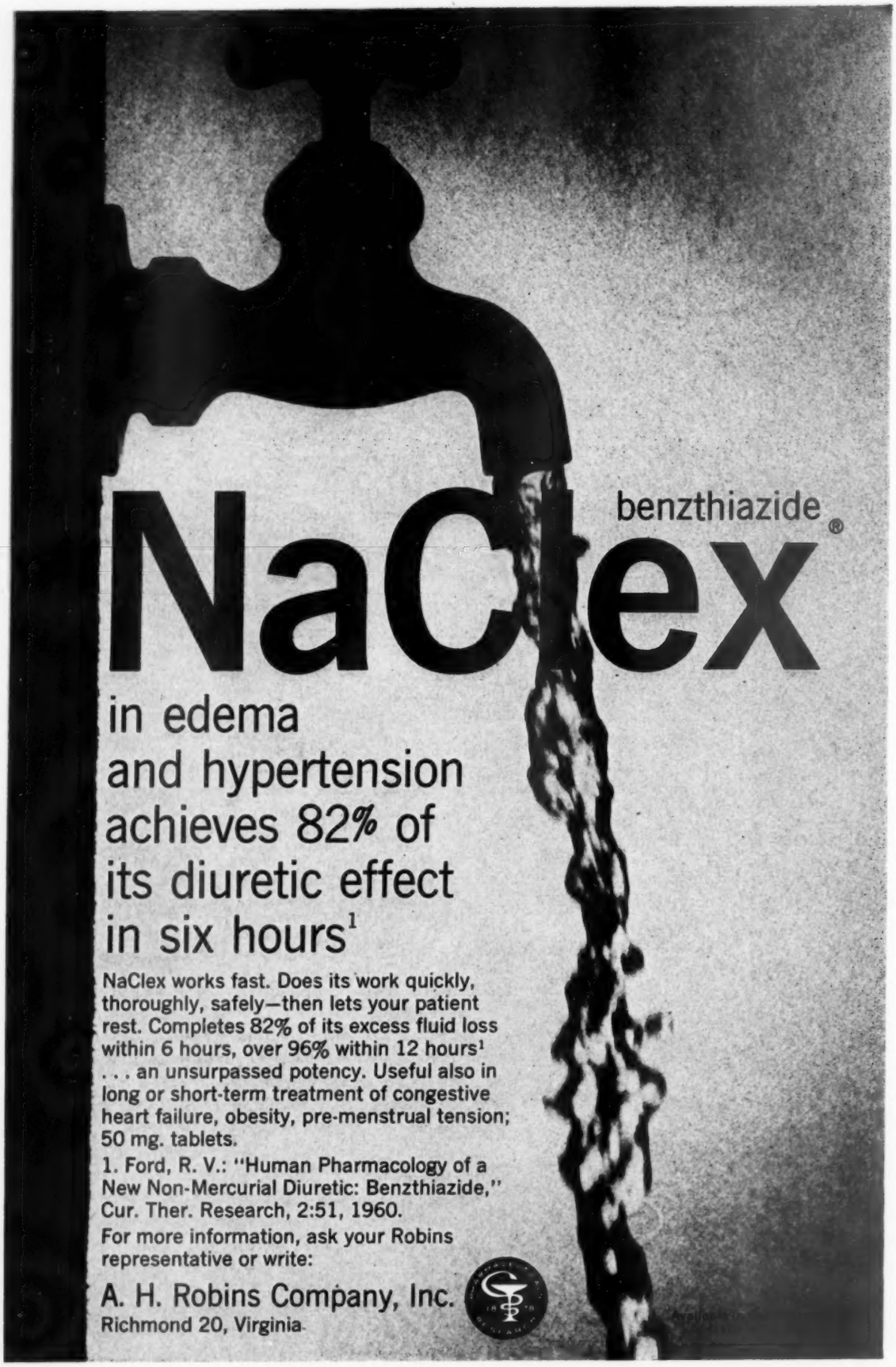
PARKE, DAVIS & COMPANY, Detroit 32, Michigan


benzthiazide®
NaClex
in edema
and hypertension
achieves 82% of
its diuretic effect
in six hours[1]
NaClex works fast. Does its work quickly, thoroughly, safely—then lets your patient rest. Completes 82% of its excess fluid loss within 6 hours, over 96% within 12 hours[1] . . . an unsurpassed potency. Useful also in long or short-term treatment of congestive heart failure, obesity, pre-menstrual tension; 50 mg. tablets.
1. Ford, R. V.: "Human Pharmacology of a New Non-Mercurial Diuretic: Benzthiazide," Cur. Ther. Research, 2:51, 1960.
For more information, ask your Robins representative or write:
A. H. Robins Company, Inc.
Richmond 20, Virginia

NEW

for

FATIGUE

Spartase

physiologic anti-fatigue agent

Wyeth

SERVICE TO MEDICINE

NEW

physiologic agent
for many cases of

FATIGUE

Spartase

TABLETS

Wyeth®
SERVICE TO MEDICINE

Fatigue . . . tiredness . . . weariness . . . are common patient complaints. SPARTASE, a new anti-fatigue agent, may now help you manage many of these cases.

Counters fatigue naturally . . . with high order of safety

SPARTASE provides *natural*, *physiologic* treatment for fatigued patients. It may be used either alone in functional disorders or, adjunctively, in the presence of organic disease.

Not a CNS stimulant, enzymatic inhibitor or antidepressant.

In selected cases, SPARTASE restores normal work capacity.

Tablets

SPARTASE®

Potassium and Magnesium Aspartates, Wyeth

OFFICIAL BROCHURE

SPARTASE is a mixture of equal amounts of the potassium and magnesium salts of aspartic acid. Pharmacological and clinical observations have been made which indicate that SPARTASE participates in intermediary metabolism in such a fashion as to be effective therapeutically in the management of fatigue.

General Pharmacological Properties—The IP and PO LD_{50} values for SPARTASE in rats are 4 and 19 grams/kg., respectively.

The pharmacological activity of aspartic acid has been the subject of numerous publications[1-8] and need not be reviewed.

Laborit *et al.*[9,10] studied the effects of the combined K and Mg aspartates on groups of white rats subjected to the standard swim test. It was found that duration of swim after this therapy was significantly prolonged over that achieved with other regimens attempted. After a standard rest period of 2½ hours, the aspartate-treated animals again swam longer than any other group.

Plasma ammonia levels were measured in groups of rats similarly exposed to swim effort and drug therapy. Increase in ammonia levels noted in the controls[11] was not seen in the group pretreated with the aspartates.

A group of 16 dogs breathing a mixture of 90% oxygen and 10% CO_2 was given the combined salts of aspartic acid parenterally. Plasma and expired CO_2 tension decreased, and plasma urea concentration increased immediately[12].

The administration of K and Mg aspartates to athletes demonstrated a positive effect on neuro-muscular irritability, a significant reduction in existing fatigue and a significant prophylactic effect against the induction of fatigue[10,13,14].

Indications—The use of SPARTASE for the treatment of fatigue is not intended to supplant specific treatment for accompanying organic disease or to substitute for specific indications for potassium.

SPARTASE has a wide range of clinical utility in the management of the fatigue syndrome. It may be used effectively in the management of many fatigue problems, whether or not associated with functional or organic disease. SPARTASE is particularly useful in treating the tired patient with no evidence of organic dysfunction.

Dosage and Administration—The adult dose of SPARTASE is two 500 mg. tablets after the morning and evening meals. Approximately four days therapy are required before subjective clinical improvement may be noted; it is suggested that SPARTASE administration be continued for at least two weeks before the patient is re-evaluated.

Contraindications and Side Effects—Nausea, abdominal discomfort and diarrhea have been noted occasionally. These symptoms may be minimized by proper administration of dose after meals.

There are no known contraindications to SPARTASE therapy.

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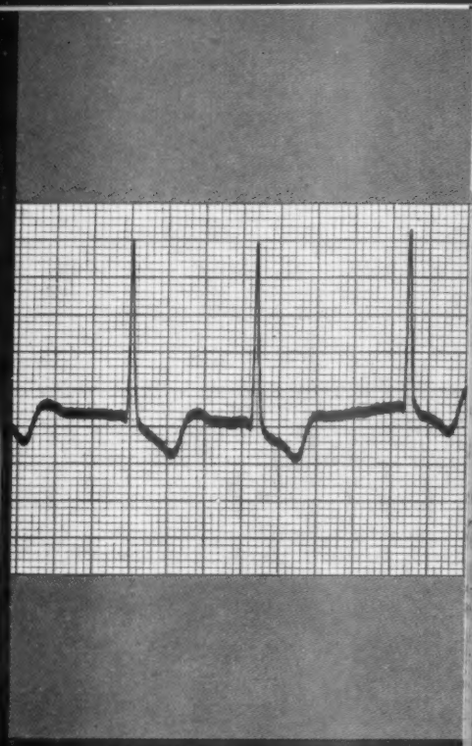
*before treatment**

Hypertensive cardiovascular disease, Grade IV (New York Heart Assn. classification), 61 year old female with atrial fibrillation, receiving maintenance dosage of digitalis.

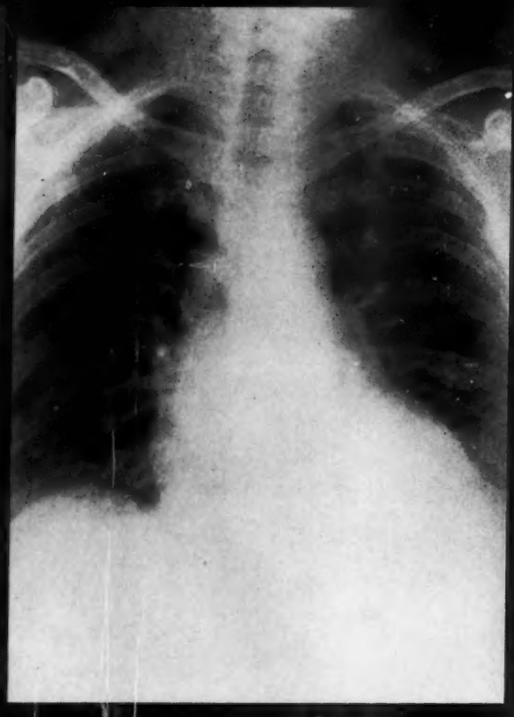
Cardiac enlargement and pulmonary congestion.



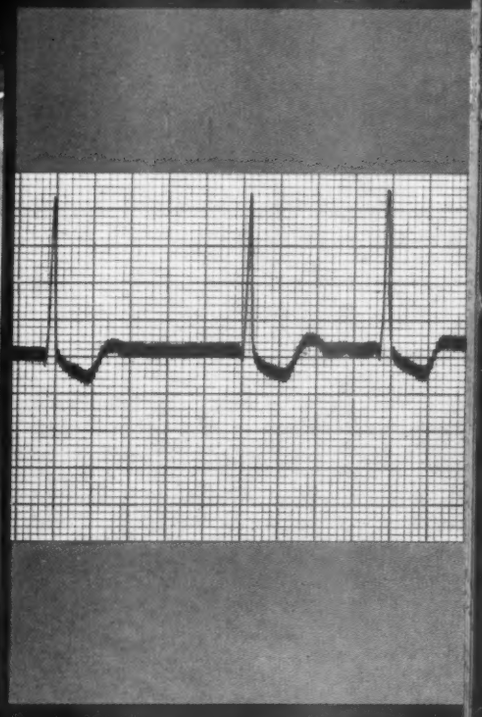
Left ventricular strain and hypertrophy (ST depression in Lead V4).

after one month on HYDROPRES*

Alternating daily dosage of 1 to 2 HYDROPRES 50 Tablets



Reduction in heart size and clearing of congestion.



Changes toward normal (less ST depression).

*case report

effective by itself in many hypertensives...
indicated in all degrees of hypertension

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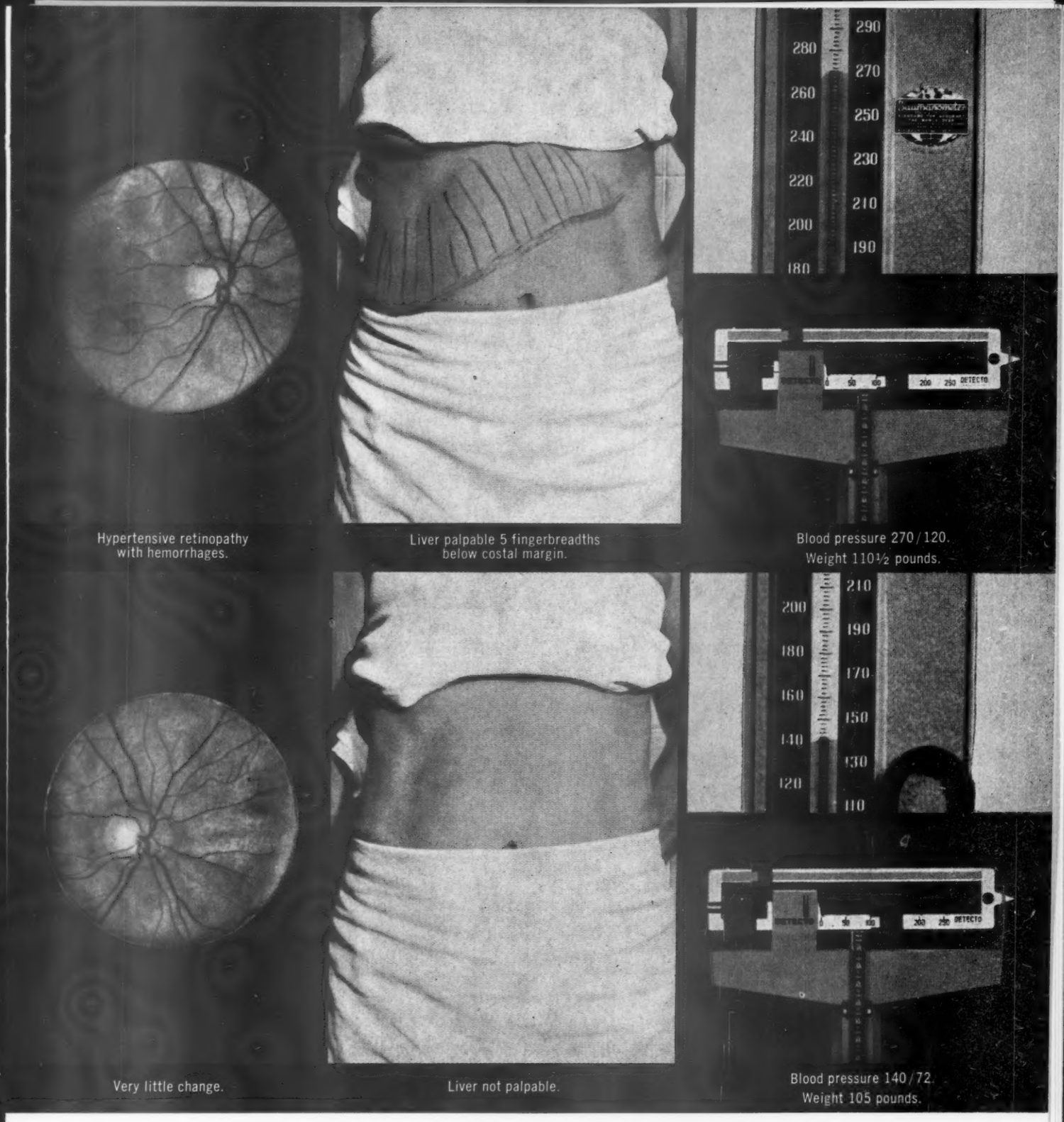
HYDRODIURIL® HYDROCHLOROTHIAZIDE with RESERPINE



Hypertensive retinopathy with hemorrhages.

Liver palpable 5 fingerbreadths below costal margin.

Blood pressure 270/120.
Weight 110½ pounds.

Very little change.

Liver not palpable.

Blood pressure 140/72.
Weight 105 pounds.

HYDROPRES-25

25 mg. HydroDIURIL hydrochlorothiazide, 0.125 mg. reserpine per tablet. One tablet one to four times a day.

HYDROPRES-50

50 mg. HydroDIURIL hydrochlorothiazide, 0.125 mg. reserpine per tablet. One tablet one or two times a day.

also available:

HYDROPRES-Ka†25

25 mg. HydroDIURIL hydrochlorothiazide, 0.125 mg. reserpine, 572 mg. potassium chloride (equivalent to 300 mg. potassium) per tablet.

HYDROPRES-Ka†50

50 mg. HydroDIURIL hydrochlorothiazide, 0.125 mg. reserpine, 572 mg. potassium chloride (equivalent to 300 mg. potassium) per tablet.

It is essential to reduce the dosage of other antihypertensive agents, particularly the ganglion blockers, by at least 50 per cent immediately upon addition of these agents or of HYDROPRES Tablets to the regimen.

Before prescribing or administering HYDROPRES, the physician should consult the detailed information on use accompanying the package or available on request.

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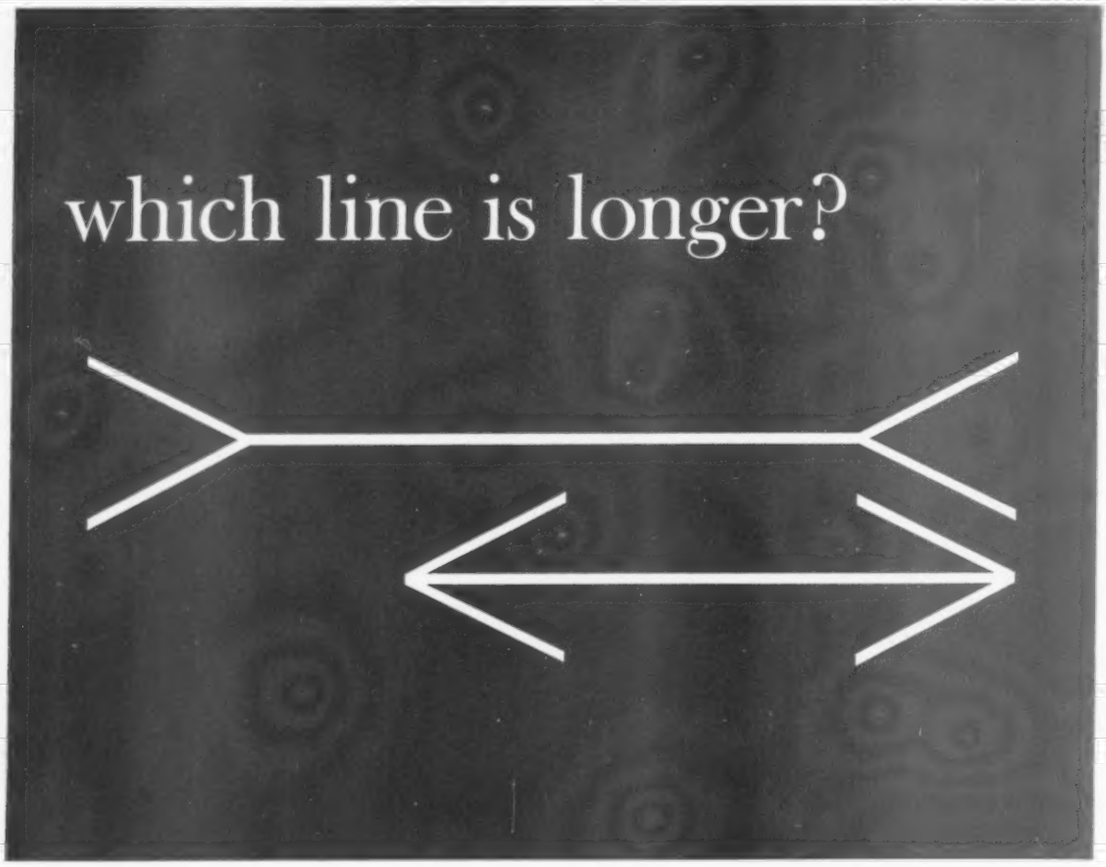

A familiar illusion. Actually, of course, the horizontal lines in both figures are the same length. And yet, doubt lingers even after measurement is made.

Take the comparison of two oral penicillins as another example. If only the price of the drugs were to be considered, the choice would be clear. But isn't it what a drug *does* that counts?

V-Cillin K® achieves two to five times the serum levels of antibacterial activity (ABA) produced by oral penicillin G.[1] Moreover, it is highly stable in gastric acid and, therefore, more completely absorbed *even in the presence of food.* Your patient gets more dependable therapy for his money . . . and it's therapy —not tablets—he really needs.

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V-Cillin K® (penicillin V potassium, Lilly)
1. Griffith, R. S.: Antibiotic Med. & Clin. Therapy, *7*:129, 1960.

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Editorial

"Propetology," A New Branch of Medical Science?*

WHILE it has been known in a general way since the time of Hippocrates that "different sorts of people have different maladies," medical science has not been in a position until recently to develop expertness in determining either what different "sorts" of people exist, or what maladies are characteristic of the different "sorts."

We deal with (acquired) resistance to infectious diseases in the science of immunology, but a new science appears to be aborning which will deal with innate susceptibility and resistance primarily to diseases of a non-infective nature. This new branch of medical science may be called *propetology* (Gr. propet, leaning toward).

The tools and the impetus to develop expertness in determining, in advance, what people are prone to contract any specified disease are now at hand; the new science needs only to be developed.

One of the obstacles which has stood in the way of the earlier development of this science is what seems to us to be an immature view of heredity and life. In medical circles there has often been the attitude "I must not admit to myself and reflect the idea to my patients that their troubles have any genetic roots; otherwise I will appear to be 'sealing their doom'." Certain groups interested in cancer, for example, will not allow statements implicating heredity in cancer to be promulgated for this reason, although every student of cancer knows that genetics and cancer must be closely interrelated.

This "escapist" point of view acts unjustifiably as a bar to development of the new science. The fact that diabetes has complex genetic roots does not prevent the use of insulin or whatever other measures may be appropriate to bring relief, prevention or amelioration. Many eye defects are hereditary in origin. This does not prevent the prescribing of appropriate spectacles. We human beings, for hereditary reasons, do not have much hair on our bodies, but this does not mean that we are doomed to be cold and shivering whenever the weather is chilly; we wear warm clothing.

A more mature view of heredity and life involves contriving, on the basis of what we know, how to overcome whatever difficulties may arise by environmental means. There are many cases in which knowledge of the "how" and "why" of heredity will pave the way toward remedial measures. One of the most recent developments in this category is that concerned with the prevention of the development of mental impairment in babies prone to phenylketonuria by the use of diets low in phenylalanine [*1,2*]. Knowledge of the hereditary basis of this disease has paved the way for its environmental management.

The same basic potentiality exists in a host of other diseases of great public interest: gout, arthritis, atherosclerosis, muscular dystrophy, multiple sclerosis, cardiac and circulatory diseases, cancers of various types and in various sites, obesity, dental caries, acne, allergies, and

* Manuscript received July 7, 1961.

even schizophrenia, alcoholism and drug addictions. An outstanding difficulty in these common diseases is that we know so little about *how* heredity is involved [3]. It must be involved, however, because there is strong evidence with respect to diseases of every type mentioned that some persons are prone and others are resistant. Environmental conditions which will precipitate the various diseases in certain prone subjects will be without effect in resistant subjects.

Probably the first scientific hints that such a science as propetology might be in the making came with the discovery of blood groups (Landsteiner, 1900) and inborn errors of metabolism (Garrod, 1902). While these findings pointed toward individual differences in body chemistry, there was no basis for supposing that these could be made the basis of disease prevention. Sheldon's definitive work on somatotyping (1940) [4] made possible a classification of people, but no claim that this classification is of more than limited value is justified, although the objective is clearly to ascertain what "sorts" of people exist.

"Constitutional Medicine," as advocated by George Draper of the College of Physicians and Surgeons, employed for diagnostic purposes any method that would yield insight into the individual patient, including anthropometric measurements, somatotyping and "over-all observation and correlation of feature detail and relationship of hair, pigment, teeth and joints" [5]. A consideration of the various writings of adherents to this school of thought emphasizes the importance for diagnostic, if not for prognostic, purposes of considering individual differences in constitution.

It is apparent that the study of innate susceptibilities and resistances must rest upon knowledge about individuality. If individuals were all exactly alike, no differences in susceptibility could exist. As long as the known individual differences are vague and are based largely upon subjective judgments, knowledge about susceptibilities and resistances will be correspondingly vague and indefinite.

The basis for substantial advance in studying innate susceptibilities and resistances must reside in increased knowledge about individuality in the areas of anatomy, physiology and biochemistry, where reasonably accurate measures, repeated as often as may be necessary, are possible. Here we find a wealth of possibilities in a territory which is still largely unexplored [6].

Having inadequate knowledge as to the basis of the resistance possessed by the more fortunate person, we have small chance to confer this (obscure) resistance on the other. Not knowing how or why the resistant one is able to escape, we are impotent to confer resistance (or make a serious attempt to do so) on the susceptible subject.

The term "propetology" has the advantage that it emphasizes that people merely *lean toward* certain diseases; it is by no means inevitable that they will contract them. There is probably no single disease among those mentioned which might not be prevented, at least to a substantial degree, if we knew what the roots of resistance are and took appropriate measures. People who are *prone* to have arthritis or stomach cancers or to become alcoholics or mental cases would not need to suffer from the respective diseases if the proneness were recognized early and if appropriate measures (largely unexplored up to now) could be taken to overcome it.

Nutrition is one of the most highly influential environmental factors which is capable of profound alteration. Its potentialities are greatly increased by knowledge and insights which have come to the fore in recent years. From the tissue culture work of Eagle [7] and others it is clear that different cells in our bodies do not by any means have identical nutritional requirements, and of necessity certain nutrients are produced in some cells and carried by the blood to other cells which are unable to thrive without these domestic importations. It is likewise clear that all cells and tissues can be nourished at various levels of efficiency. The nourishment may be at such a level that the cells and tissues merely "exist"; it can be up to any level including that producing full vigor. Many diseases are probably caused by nutritional difficulties at a level of local tissues. Many of the experiments performed or contemplated attempting to use nutrition to combat various disease conditions are naive and unsophisticated in the light of the facts of cellular nutrition [8].

When propetology develops, it will be found, in all probability, that various types of proneness can be overcome by careful attention to the nutrition of the prone person. Instead of consuming a diet thought to be applicable to everyone, the person who is prone to contract a particular disease may have to avoid some food items (as in the case of phenylketonuria) or concentrate on some other items, or he may have to have his diet modified by the addition of

specific nutrients. The potentialities of scientifically planned nutrition to overcome proneness are great, but they cannot be realized until propetology develops.

The route by which this new science will develop seems relatively clear in broad outline. First there must be collected a vast and cumulative amount of data regarding individuals and their distinguishing characteristics [*9*]. Tables which merely yield norms, means and standard deviations, and the like, are without value in this connection. The type of information most pertinent in connection with one disease may be almost immaterial so far as another disease entity is concerned. Therefore it seems likely that an attempt to encompass all diseases in one study would be ill advised.

It should be emphasized that research into the biochemical, physiologic and anatomic basis of disease susceptibility will not involve merely seeking "abnormal" values. The kind of norm which, applied to one parameter, includes most of the values exhibited by the general population is of little or no value in this connection. Major attention will be directed to values within such a "normal" range because most members of the general population exhibit disease susceptibility. Disconformities [*10*] will demand attention and each person's "norm" will, at least to a degree, be determined.

Propetology will achieve success through studies of measurement trends, intra-individual variation, and analysis of complex patterns of measurements [*11*], as well as by the traditional methods of searching out single significant items and two-variable correlations.

For the development of this science major attention should be directed to the development of new measurements of potential prognostic value. Such investigations have lagged behind the advance of basic biochemistry and physiology. Too often we think of using simple, convenient, standard tests even though their clinical or prognostic value is extremely dubious. The rapidly advancing area of instrumental automation should make feasible the rapid expansion of our repertoire of prognostic research technics.

It is most interesting and significant from the standpoint of this discussion that an experiment is being planned at the National Institutes of Health which involves carrying out a series of tests on 500 young people (who are now well), repeating the tests from time to time over a period of years, paying particular attention to the types of maladies which afflict them [*12*]. This will be a pioneer study in the area of "propetology." It involves the use of a new methodology and strategy, inasmuch as individual patterns will be studied, and the concept of normality will be practically if not wholly disregarded.

Practicing physicians can be assured, especially if they lend moral support, that the science of propetology will be developed in many laboratories, and that as a result the management of many diseases, may, in the not too distant future, be more successful.

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Austin, Texas

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Clinical Studies

A Third Type of Periodic Paralysis, with Normokalemia and Favourable Response to Sodium Chloride*

DAVID C. POSKANZER, M.D.† *and* DAVID N. S. KERR, M.B., M.SC.
Newcastle upon Tyne, England

ALTHOUGH fewer than 1,000 cases of periodic paralysis have been recorded [*1–3*], the disease has excited interest out of proportion to its clinical importance because of the insight it may reveal into the metabolism of electrolytes and muscle. Since the demonstration in 1934 by Biemond and Daniels [*4*] that a low serum potassium level accompanies some attacks, the literature has burgeoned. More than twenty-five theories as to etiology have been presented [*5*], and confusing and often contradictory literature has accumulated. It soon became apparent that the change in serum potassium was not the basic defect in the disease. Not only do some patients show no alteration in serum potassium levels and no response to the administration of potassium, but also when serum potassium levels are low they are still within limits which fail to produce weakness in normal subjects or patients with renal potassium loss.

A number of investigators have recognised that the syndrome of periodic paralysis may be produced by several different mechanisms [*6–8*]. It is possible that all are due to a single as yet undiscovered pathologic mechanism, but the differences in potassium metabolism render this hypothesis unlikely. Not only are families reported in which affected members have a low serum potassium and diminished urinary excretion of potassium during attacks, but families have also been reported with *increased* serum potassium levels during attacks. The latter syndrome has been extensively studied by Gamstorp [*3*] under the title adynamia episodica hereditaria.

Classic periodic paralysis is characterized by low serum potassium levels during attacks, diminished urinary excretion of potassium, rapid response to orally administered potassium salts, and precipitation of attacks by several manoeuvres which lower serum potassium levels, including carbohydrate feeding, injection of insulin and adrenalin, and the administration of sodium-retaining, potassium-excreting hormones such as 2-methyl-9-alpha-fluorohydrocortisone. Onset is usually in the second decade of life. Attacks last from one hour to four days, averaging twelve hours, and occur about once a month or two. The patient usually awakens paralyzed in the morning. (Table I.)

The two families which Gamstorp described as having adynamia episodica hereditaria included 138 patients with spontaneously abating attacks of paralysis, usually occurring during rest after exertion. The onset of illness was in the first decade of life. Attacks occurred at least once a week in three-fourths of the cases and lasted less than an hour. They were relieved by gentle exercise or the intake of food. Attacks were more common in the daytime, and were equally prevalent in both sexes but were more severe in males. Increased serum potassium levels were noted in forty-five of forty-nine observed attacks. There was no decrease in urinary excretion of potassium as an attack developed. (Table I.)

Tyler, Stephens, Gunn and Perkoff [*6*] described a family with normal serum potassium levels during attacks. The group consisted of

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† Moseley Traveling Fellow of Harvard University. Present Address: Department of Neurology, Massachusetts General Hospital, Boston, Massachusetts.

TABLE I

COMPARISON OF THREE TYPES OF PERIODIC PARALYSIS

	Classic Periodic Paralysis	Hyperkalemic Adynamia Episodica Hereditaria	Sodium Responsive Normokalemic Periodic Paralysis
Onset	Second decade (puberty)	First decade	First decade
Frequency	Every one to two months	Usually weekly	Every one to three months
Duration	One hour to four days (average twelve hours)	Less than one hour	Two days to three weeks
Serum potassium	Low	High	Normal
Provoking factors	Rest after exertion, overeating, (carbohydrates), cold, mental tension, alcohol	Rest after exertion, cold and damp	Rest after exertion, sitting still, sleeping late, alcohol, cold and damp, mental stress
Iatrogenic provocation	Adrenalin, glucose and insulin, sodium chloride, fluorohydrocortisone	Potassium chloride	Potassium chloride
Severity	Complete paralysis sparing face and respiration	Seldom complete	Often complete, including jaw, and cough reflex
Time of day	Awaken paralyzed	Often diurnal	Awaken paralyzed
Sex difference	More severe in males	More severe in males	Similar in both sexes
Sensory changes	Nil	Parasthesiae	Peripheral hypaesthesiae (one case)
Metabolic changes	Potassium retention, ? sodium retention	No retention of sodium or potassium	Potassium retention
Prophylaxis	Spironolactone	Gentle exercise after exertion	Gentle exercise after exertion
	Very low sodium diet	Carbohydrate intake, acetazolamide	Fluorohydrocortisone, acetazolamide
Treatment of attack	Potassium chloride	Calcium	Sodium chloride

thirty-three subjects with recurrent attacks of weakness or flaccid paralysis lasting from a few minutes to several days, beginning in the first year of life. A heavy meal, particularly if taken before bedtime, was sometimes followed by an attack the next morning. Some extensor muscle weakness was present even during "normal" periods, and enlargement of muscles out of proportion to their strength was noted. No correlation of strength with serum potassium levels could be found. Vacuolization of muscle fibres was present in biopsy material.

McArdle [7] studied a similar patient with a slightly raised potassium level during weakness, which fell on recovery. This patient had an increased excretion of potassium during the paralysis, in contrast to the low excretion levels in classic paralysis.

Our own interest in the subject of periodic paralysis was stimulated by the successful treatment of a patient with the classic type with spironolactone [9]. In an effort to make this treatment available to other patients with the disease in the North East of England we attempted to locate all the patients through hospital records. The area served by the two neurologic consultants in Newcastle upon Tyne contains 2,300,000 people. In this population we were able to locate four families suffering from the disease, with twenty-one affected members still living. In the course of examining these patients we were surprised to find that they included only three cases of periodic paralysis of the classic type and two cases of adynamia episodica hereditaria, both under study by McArdle. The remaining patients were members of a single family, and in part resembled each of the previous two varieties but seemed to constitute a third and separate type.

THE SOUTHWOOD-GANNON FAMILY

Three members of the Southwood-Gannon family were investigated by the late Sir James Spence thirty years ago as having "periodic paralysis" before the changes in serum potassium had been described. Figure 1 illustrates the family tree, and Table II summarizes the data illustrated. The eldest forebear (Case I, Thomas

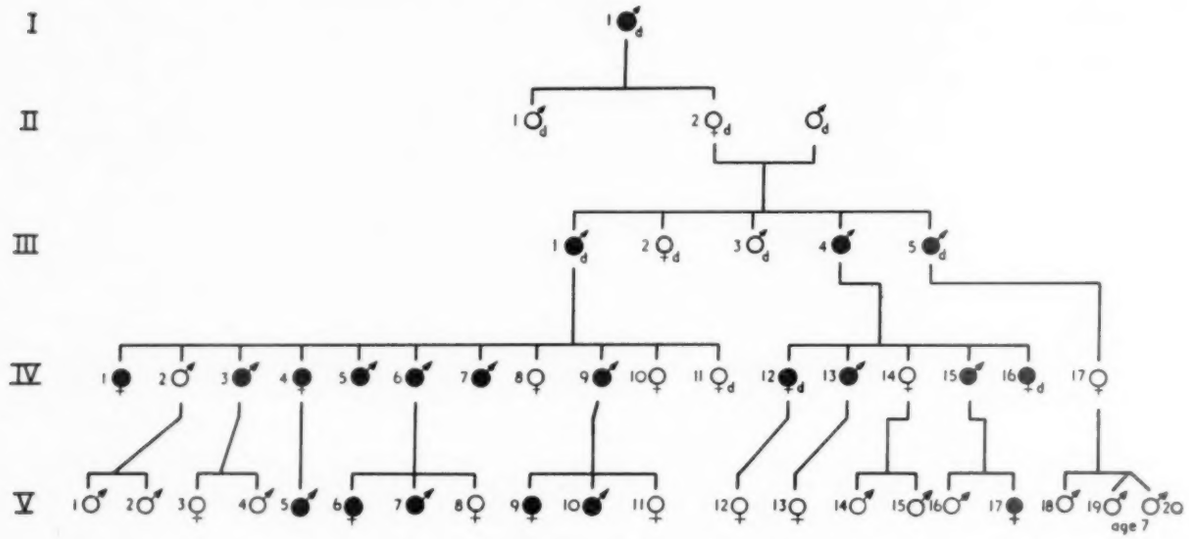

FIG. 1. The Southwood-Gannon family.

Southwood) moved to the Newcastle area from West Cumberland over 120 years ago. All persons illustrated in the family tree are over the usual age of onset of the disease in the first decade, with the exception of two (Cases v 19 and v 20) who are only seven. In the others the affliction could reasonably be expected to have become evident. No person in the family died prior to the age of fifteen. Only affected parents transmitted the disease to their offspring except in one instance (Case II 2), the forebear of the main family line who is reported not to have suffered from the disease. Information about her, however, is limited. She died at forty-four and no living member of the family remembers her well enough to state whether or not she had attacks.

TABLE II
SEX DISTRIBUTION OF AFFECTED AND NORMAL MEMBERS OF THE FAMILY

Generation	Total Members		Affected Members		Normal Members	
	Male	Female	Male	Female	Male	Female
I	1	...	1	...	...	...
II	1	1	...	...	1	1
III	4	1	3	...	1	1
IV	8	9	7	4	1	5
V	12	8	3	3	9	5
	26 (100%)	19 (100%)	14 (54%)	7 (37%)*	12 (46%)	12 (63%)
Total	45 (100%)		21 (47%)		24 (53%)	

* The occurrence of the illness in only 37 per cent of females is not significant. P is greater than 0.3 that this is a chance occurrence.

With the exception of this single instance the family pattern is compatible with autosomal dominant inheritance with complete penetrance. There is no history of consanguinity so that affected members of the family are almost certainly heterozygous. The chance of an affected member of the family passing on the trait is therefore one in two. In four instances an affected parent had two or more offspring who were affected. In each of these instances the affected offspring were of both sexes. Sex-linked and partially sex-linked inheritance are therefore ruled out.

Clinical Features. Clinical information is available for twenty-one patients in the Southwood-Gannon family. Personal clinical histories were obtained from fifteen of twenty-one patients. Five members of the family who had suffered from this disability were dead and one had moved out of the area. In these six, the clinical histories were not taken directly from the patient but from a sibling, and may tend to emphasize severe episodes of disability. Most of the patients also tended to ignore less serious bouts of weakness unless specific enquiry was made.

Age of onset: Among the fifteen patients for whom data on age of onset were available the age of onset was two years in four, three years in two, four years in two, five years in three, and from six to ten years in four.

Severity and frequency of attacks: Twenty of the twenty-one patients about whom clinical information is available have at some time in their life had one or more severe attacks of paralysis,

during which they were quadriplegic, had to be turned in bed, and often had facial weakness, difficulty in chewing and weakness of cough. Complete paralysis usually lasted a week, and one to two additional weeks were required for gradual recovery. In several instances complete paralysis, during which the patient had to be turned in bed, lasted from two to three weeks.

None has had such severe attacks more often than once in two months. Eleven patients reported such attacks once in two to four months, five have had them once or twice each year, and three have had only one to three such attacks since the onset of illness. One patient has never had such a severe attack. Another patient, now dead, had moderate attacks but it is not known whether he was subject to severe attacks.

Eight patients also reported moderately severe attacks, during which they are unable to walk or are severely impaired in activity, and have generalized weakness but not complete paralysis for two to four days. These attacks occur once every one to three months. During these episodes the patients cannot work.

All the patients complained of prolonged residual weakness at times between severe episodes, often dragging on for several weeks. During these periods they are able to carry on only at a reduced level of activity. Shorter mild attacks are unusual. Once an attack of weakness begins to develop it usually proceeds to moderately severe disability. Only three patients reported momentary weakness of one or two limbs, lasting up to twenty minutes and then clearing.

Depression and irritability during the attacks were reported by many patients. Bladder and bowel function are never impaired. In one patient only was sensory loss of a stocking type up to the knee in one leg found to be present on hospital examination during an attack. The patient was unaware of this deficit and it disappeared only to return with each of three successive bouts of observed weakness. During one very severe attack demonstrable hypaesthesia was present in all four limbs. Parasthesias of the face early in attacks, described by Gamstorp in patients with adynamia episodica hereditaria, did not occur in our patients. Paralysis is often asymmetrical and often affects the parts most used, the legs of a cyclist or the arms of a house-painter.

There was little agreement among the older patients about the age of most severe disability. Two were of the opinion that the attacks were less severe after thirty, one after forty-five and one after sixty. Two believed that the illness was at its peak in the second decade of life but one considered that his worst years had been between forty and fifty. In most cases the patients seem to learn to adjust to the disability over the years and avoid the circumstances which tend to precipitate attacks.

Precipitating factors: Attacks of paralysis are precipitated in a number of apparently unrelated ways, including rest after exertion, sitting or standing in one place for a prolonged period, sleeping late in the morning, alcoholic intoxication with vomiting, cold and damp, and severe mental stress.

Most commonly, rest after strenuous exertion brings on weakness. More than a single factor is involved in precipitation of an attack, however, for similar degrees of exertion do not consistently cause attacks. All of the affected family members report that severe exertion may result in an attack but if they walk about or exercise gently after strenuous effort they can avoid paralysis. The patriarch of the family, now seventy-seven years old, can remember an episode of paralysis overtaking him at his work as a pitman in the Northumberland coal mines where, because of the narrow coal seams, he spent most of the working day on his knees. Although he was able to rise to leave the pit, the paralysis was progressive and he crawled the final mile to his home on hands and knees across the Newcastle town moor.

Sitting or standing still for a prolonged period predisposes to attacks in many members of the family. One student has some weakness one day of each week when he sits through four consecutive hours of classes. Several members of the family will not attend a double feature cinema programme although they tolerate single films. The additional time spent sitting through the second motion picture is often sufficient to set off an episode of weakness the next day. Standing in one place for two hours, a common custom for spectators at British football matches, often initiates weakness.

Sleeping late in the morning is second to exertion as a factor in precipitating attacks. This is true to such a degree that the family often refer to the disability as "Sunday morning paralysis." Such attacks occur in members who usually arise early and who spend an extra

three hours in bed on Sunday morning. One or two extra hours will not usually produce an attack, but three extra hours in bed will regularly bring on weakness in some patients.

Many patients reported episodes of paralysis on the morning after excessive alcoholic intake, usually beer, followed by vomiting. In one such patient an attempt was made to determine whether or not the paralysis was related to the electrolyte loss as a result of vomiting. These findings are detailed under "Metabolic Studies." Apparently the vomiting is not in itself the important precipitating factor, nor was severe diarrhea implicated by any of the patients. Two patients had had episodes of diarrhea with over twenty movements a day without resulting paralysis. In no instance has the ingestion of a heavy meal or carbohydrate intake been implicated, either in the production of an attack or in the warding off of an impending paralysis.

Cold and damp weather and exposure favour the onset of attacks, and several patients reported attacks of paralysis brought on by severe emotional stress. One patient had her first attack at the age of three during a bombing raid on London. Although frightened during the raid, the patient was unhurt. After the all-clear, she was found to be quadriplegic and was admitted to hospital as a possible case of poliomyelitis. Her parents were able to persuade the medical authorities that the illness was a familial paralysis and a common occurrence in members of the family. The patient's complete recovery in four days supported that impression.

Extent of disability: All members of the family lead active useful lives and accept the disability when it occurs as part of the normal course of life. The usual pattern is either a severe or complete paralysis, a moderately severe bout sufficient to prevent the patient from working but with retention of some movement, or several weeks of moderate weakness during which the patients carry on as best they can. Each affected member of the family, including affected females, has a job or is attending school.

Three of the males had served in the Armed Forces. One was given a medical discharge after an episode in which he was accused of malingering by his sergeant because he awoke one morning paralyzed and was unable to get out of bed. One patient was transferred to the catering corps where his disability was less important. One continued to serve in the peacetime army but since the age of twenty he has had no severe attacks, only mild episodes of weakness lasting several days.

CLINICAL HISTORIES

CASE 1. W. G., a fifteen year old schoolboy, has had attacks of paralysis since the age of ten. On the first occasion after sitting in one place for an hour and a half his left leg became "stiff" and felt numb. "Stiffness," which connotes both weakness and excessive firmness of the tissues, began in the calf and spread to involve the thigh and the entire limb. Disability lasted a week.

Since then he has an attack about once a month during which the legs stiffen and become weak. In most attacks weakness is maximal on awakening on the first or second day and rarely increases during the day. Occasionally there is a further upward progression to involve the arms on the second or third day. These episodes are usually precipitated by exercise followed by rest. Once or twice a year the patient has a major attack beginning with weakness of the legs but followed on awakening next day by almost complete paralysis. On these occasions he is helpless and has to be turned in bed. Swallowing, speech, breathing, bladder and bowel function are intact. The muscles of mastication are sometimes weak and cough force is diminished. The weakness regresses in reverse order, leaving first the arms and trunk, then the legs. Complete paralysis lasts one week, and an additional ten days to two weeks are required before function is normal.

On examination during an attack the muscles of both legs were hard and tender at first but became flaccid as paralysis became more marked. Reflexes were reduced or absent. There was a diminution of sensation to pin-prick and temperature discrimination, in a stocking distribution over the lower left leg up to the knee, which appeared only with weakness and disappeared as strength improved. In one severe attack observed, pain and temperature sensation was impaired peripherally in all four extremities. Touch vibration, joint position sense and two point discrimination were normal.

Physical examination is entirely within normal limits between attacks. At no time was there evidence of myotonia in limbs or tongue. There is no residual weakness or pseudohypertrophy.

Roentgenograms of the skull and chest did not reveal any abnormalities. Roentgenograms of the lumbar spine showed a spina bifida of the sixth vertebra of the transitional type. Blood count, urinalysis and sedimentation rate were all within normal limits. Urinary excretion of amino acid was 336 mg. per twenty-four hours. Fasting blood sugar was normal. Pyruvic acid during an attack was 2 mg. per 100 ml. (normal is less than 1 mg.). The blood urea nitrogen was 27 mg. per cent. Creatinine clearance calculated during an attack was 160 L. per twenty-four hours and during a normal period 185 L. per

twenty-four hours (both normal). Serum calcium and phosphate levels were within normal limits both during attacks and normal periods, but plasma phosphate levels were raised (5.6, 5.9 mg. per cent) on two occasions. The serum total proteins were 8.6 gm. per cent (normal 6 to 9.3 gm. per cent, ultraviolet absorption method). Information derived from electromyography and muscle biopsy is discussed under "Special Studies of Muscle."

After strenuous exercise followed by rest the patient became moderately weak and walked with a limp. He was given 12 gm. potassium chloride which resulted in total paralysis of the lower extremities, weakness of arms and inability to sit up unassisted. The results of electrolyte studies made during this period will be discussed subsequently.

A second, more serious attack of paralysis occurred when the patient's prophylactic medication was discontinued. After he had avoided attacks for two and a half months on a regimen of acetazolamide, 250 mg. daily, and 2-methyl-9-alpha-fluorohydrocortisone, 0.1 mg. daily (see discussion of therapy to follow) the administration of these medications was discontinued. No effect was observed for three days. On the fourth day the patient, who is known to consume a large quantity of salt daily, was asked to avoid taking any added salt with his meals. The following day he awoke partially paralyzed and on the sixth day after discontinuation of medication was found to be quadriplegic on awakening, with weakness of the neck and muscles of mastication and a diminished cough. He had to be fed and turned in bed. In addition, glove and stocking loss of pain and temperature sensation was present. The extremities were cold and produced an excessive flare reaction when a pin was dragged over the hypaesthetic parts. Reflexes were absent in all four extremities. The cranial nerves, including facial sensation, were intact save for the muscles of mastication. General physical examination was within normal limits. Paralysis and hypaesthesia remained unchanged for seven days. Recovery then occurred gradually over a three-week period.

Case 2. G. G., a seventeen year old insurance clerk, had his first episode of paralysis at the age of two when he fell off a chair and complained that he felt weak. Since that time he has had about one really severe attack per year, two moderately severe ones per year, and a mild attack monthly. During a severe attack he is completely paralyzed and has to be turned in bed. There is difficulty with chewing but no impairment of breathing or alteration in facial expression. He is usually bedridden for a week and recovers complete strength after an additional week.

A moderately severe attack usually involves general weakness, most marked in the legs. He has noted an episode of this sort after sitting quietly in school for several hours. On standing, he then drags his left leg behind him when walking. This difficulty may last just a few hours or through the entire day. With moderate weakness the patient notes a stiffening of all muscles, whereas with a severe attack they become flaccid. During an attack the left arm is slightly worse than the right. Between attacks physical examination is within normal limits. There is no evidence of myotonia.

Precipitating factors include sitting quietly for any length of time, sleeping several extra hours on Sunday morning, which will often precipitate a really severe attack of weakness, and sitting down after strenuous exercise. The patient walks for several minutes after exercise to avoid paralysis. On one occasion following food poisoning the patient was well on awakening, vomited copiously once, returned to bed and was severely paralyzed several hours later.

Roentgenograms of the chest and lumbar spine were within normal limits. Blood urea nitrogen was 25 mg. per cent, and sodium 143 mEq. per L., potassium 3.7 mEq. per L., chloride 110 mEq. per L., bicarbonate 28 mEq. per L. in a specimen drawn at home during an episode of weakness.

Case 3. B. G., a nineteen year old laboratory technician, had his first episode of weakness at the age of six when he noted stiffness and weakness in both legs lasting for two to three days. Although additional attacks occurred he had little serious difficulty until the age of seventeen when, after drinking 7 pints of ale, he became intoxicated and vomited during the night. On awakening the following morning he found his legs were completely paralyzed, his arms very weak and he was unable to sit up. Speech, cough and swallow, and bladder and bowel function were normal, but the muscles of mastication were weak. This disability remained static for three days, then gradually began to clear over the ensuing seven days. He was able to stand after one week and returned to work one week later.

He has had four similar attacks since, all precipitated by excessive alcoholic intake with vomiting. Milder attacks also occur, lasting four to five days and affecting only the legs, during which the patient is still able to move about. These milder episodes have occurred every two to three months. The patient is of the opinion that weakness begins in the thighs and travels distally. He has been treated in the past with potassium chloride orally but thought that it made the weakness worse rather than better.

General physical examination and neurologic examination were entirely within the limits of normal. There is no weakness between attacks and there is no evidence of pseudohypertrophy.

Blood counts, urinalysis, erythrocyte sedimentation rate and chest roentgenograms were all within normal limits. Electrolytes, when muscle strength was normal, were sodium 137 mEq. per L., potassium 3.5 mEq. per L., chloride 102 mEq. per L., HCO_3 25 mEq. per L., urea 31 mg. per cent. Urinary excretion of amino acids

was 325 mg. per twenty-four hours and 427 mg. per twenty-four hours. Creatinine clearances were 226 L. per twenty-four hours and 198 L. per twenty-four hours.

The patient was admitted to hospital on two occasions for study. During the first admission an attempt was made to induce paralysis by the ingestion of 6 pints of beer in two hours. Paralysis did not occur, probably because the patient retained only a small portion of the total intake. At the same admission moderate paralysis was achieved by the oral administration of potassium chloride. Weakness was present on awakening the morning following administration of 295 mEq. potassium.

On a second occasion the patient was admitted following a large alcoholic intake and quadriplegia developed the next day. Details of these admissions are presented under "Metabolic Studies."

METABOLIC STUDIES

It has been difficult to persuade patients to remain in the hospital for the time necessary for adequate metabolic study because all members are active and have jobs or attend school. Seven episodes of paralysis or weakness have been observed and investigated in the hospital and several additional attacks have been observed and treated in the patients' homes.

Precipitation of Attacks. By ingestion of alcoholic liquor: One attack followed a spontaneous drinking bout, during which the patient (Case 3) ingested 5 pints of amber ale, 2 glasses of sherry and 5 gins-and-tonic (total approximately 3 L. fluid, 56 mEq. potassium and 20 mEq. sodium). He was admitted to hospital in a severely intoxicated condition immediately after closing time, vomited once (450 ml.) during the night and awoke the following morning with quadriparesis and inability to sit up in bed. Plasma electrolytes were estimated on admission and during recovery from the attack.

An attempt was made to induce a similar attack under observation in hospital. Over the course of two hours the patient and a control subject each consumed 6 pints of brown ale, providing a fluid alcohol electrolyte content approximately the same as the assorted drinks previously ingested. Both the patient and control subject became moderately intoxicated but the patient vomited 2,000 ml. of a total intake of 3,300 ml., and no paralysis ensued. Plasma electrolytes were estimated at frequent intervals over the next twenty-four hours: there was a slight rise in plasma potassium in the control subject but no significant change in the patient.

Several possible explanations of paralysis following alcoholic intake and vomiting have been considered. Loss of fluid or sodium in the vomitus does not appear to be the mechanism since the intake as alcoholic drink was greater than the loss as vomitus on each occasion. Retention of potassium was considered more seriously in view of the other evidence (*vide infra*) that potassium retention accompanies attacks. The dose of potassium ingested (intake minus loss as vomitus) was 45 mEq. on the occasion when paralysis ensued and 30 mEq. during the unsuccessful attempt. This difference is too small to suggest a primary role for potassium, but as a check on this conclusion an attempt was made to induce paralysis in the same patient by administration of potassium without alcohol. Over the course of twenty-four hours, 300 mEq. of potassium chloride was administered in divided oral doses. Slight muscular weakness was present when the patient awoke at the end of this period but the administration of a further 150 mEq. over the next twelve hours caused no further increase in the weakness. Surprisingly, the plasma potassium level never rose above 4.4 mEq. per L.; the same dose administered to a control subject caused a rise in plasma potassium to 5.1 mEq. per L., but did not affect muscle power. The doses employed in this experiment were much greater than any that the patient could conceivably have ingested as alcoholic liquor, and the paralysis produced was much less profound. It is concluded that potassium retention is not the main mechanism of paralysis following alcoholic excess. The effects of water alone and of pure alcohol have not yet been investigated.

By potassium chloride administration: In addition to the attack of mild weakness of the legs following the intake of massive doses of potassium chloride already referred to, one attack has been observed which was aggravated by potassium therapy. Early in the course of our studies we administered 215 mEq. of potassium chloride over twenty-four hours to a patient (Case 1) in the hope of shortening an attack of paralysis, starting twenty-four hours after the onset of weakness. During the period of administration the moderate weakness of both legs, present before treatment, rapidly increased and extended to severe quadriparesis. In all previous attacks the maximum degree of weakness had been reached within twelve hours of onset. We therefore believe that "treatment" with potassium

chloride was responsible for the deterioration. Similar exacerbation of attacks by medically administered potassium chloride had been noticed by four of the patients.

By rest after exercise: On five occasions patients were asked to try to reproduce their attacks under observation by taking fairly vigorous exercise and then resting. On three occasions a moderately severe attack was produced.

By withdrawal of therapy: Two attacks followed the withdrawal of 9-alpha-fluorohydrocortisone, with which the patients had been treated. A particularly severe attack occurred when one patient (Case 1) withdrew 9-alpha-fluorohydrocortisone and three days later stopped taking salt on his food (*vide supra*).

Plasma Electrolyte Changes During Attacks. At the height of two attacks precipitated by exertion alone, the plasma potassium level was 4.2 and 3.5 mEq. per L. and the plasma sodium level 143 and 145 mEq. per L. The plasma potassium level in one patient (Case 3) was 3.4 mEq. per L. on admission after his drinking bout and 3.7 mEq. per L. eight hours later when he awoke paralyzed. In the other attacks studied, the plasma electrolyte levels may possibly have been altered by the administration of substances intended to precipitate or relieve paralysis. Over sixty estimations of serum electrolytes have been made during the course of seven attacks of paralysis in three patients.

No significant trend could be demonstrated in serum potassium levels during paralysis compared with the normal state and no consistent changes in serum potassium levels occurred from the onset through the peak of an attack or toward recovery. In one patient (Case 1) quadriparesis lasted for seven days during which the serum potassium ranged from 3.5 to 4.5 mEq. per L., sodium from 138 to 145 mEq. per L. A number of serum potassium determinations during the normal unparalyzed state in the same patient ranged from 3.7 to 4.2 mEq. per L. In one patient (Case 2) no serum potassium level above 4 mEq. per L. was recorded and during the height of the episode of weakness it was 3.5 mEq. per L.

In one patient (Case 3) serum potassium levels from 3.5 to 4 mEq. per L. were recorded when muscle strength was normal. Two hours after ingesting the last dose of a total 295 mEq. (22 gm.) potassium chloride over twenty-four hours, the serum potassium was 4.4 mEq. per L. and remained at 4.4 mEq. per L. the next morning when paralysis was found to be present on awakening. However, as the weakness progressed through the day the serum potassium fell to 3.9 mEq. per L. despite the administration of an additional 67 mEq. (5 gm.) potassium chloride.

The surprisingly small changes in the serum potassium level in response to potassium loading in the patients have already been commented upon.

No consistent change was found in plasma sodium, chloride or bicarbonate levels in the cases studied.

Blood urea and phosphate levels: The blood urea level rose to a peak towards the end of each of two studied episodes of paralysis. In one attack the level rose from 21 to 60 mg. per cent, in the other from 34 to 48 mg. per cent. Renal functions in other respects, including creatinine clearance, sodium excretion, total urinary volume and amino acid excretion, were normal during the periods when the urea level was raised; protein intake was not increased. It must be assumed, therefore, that elevation in the blood urea levels is due to increased urea formation rather than an intrinsic renal lesion. The demonstration on muscle biopsy of necrotic areas within muscle tissue may well explain this finding.

On two occasions during a severe episode of paralysis, when the blood urea was 43 mg. per cent, the serum phosphate was 5.6 and 5.9 mg. per cent. The serum calcium was 9.2 mg. per cent (diffusible calcium 3.7 and 3.9). The elevated serum phosphorus levels may also be an index of tissue breakdown. The phosphate excretion index [*10*] was plus 0.095 (normal $\pm$ 0.09) one week after termination of an attack of paralysis.

Sodium and Potassium Balance Before and During Attacks. In classic periodic paralysis potassium retention precedes and accompanies attacks, and Conn and his colleagues [*11,12*] have shown that sodium retention also may precede the attack. We have had only one opportunity to study a patient from the present family during the onset of an attack not precipitated by the administration of electrolytes. During the first twenty-four hours paralysis was induced by rest after fairly severe physical exertion, reached a maximum and became stationary. Over the second twenty-four-hour period 16 gm. potassium chloride was administered in divided oral doses, and paralysis

TABLE III
SODIUM AND POTASSIUM BALANCE DURING ONSET OF PARALYSIS IN PATIENT 1 (ATTACK 1)

Period (hr.)	Electrolyte	Intake (mEq.)	Output (mEq.)	Balance (mEq.)
0–24	Sodium	43	133	−90
	Potassium	51	16	+35
24–48	Sodium	43	87	−44
	Potassium	266	27	+239

increased further. Balance data are given in Table III. The patient passed no stool during the attack; this is a common occurrence with all affected members of the family. Faecal collections were made during a later, more prolonged attack in the same patient and amounted to sodium 2 mEq. per twenty-four hours, potassium 4 mEq. per twenty-four hours. The small losses of electrolytes into the colon during attacks of paralysis with constipation must therefore be negligible; we have not attempted to measure faecal loss in other patients. "Balance" is therefore used here to indicate intake minus urinary loss.

In one patient balance studies were carried out during the production of weakness by the administration of potassium (*vide supra*). Over the forty-eight hours including potassium chloride administration and the onset and development of weakness, the potassium balance was: intake 565 mEq., output 183 mEq., balance +382 mEq. Sodium balance during the first twenty-four hours was: intake 34 mEq., output 80 mEq., balance −46 mEq. An isolated urine sample collected during the onset of another spontaneous attack of paralysis contained 184 mEq. sodium and 12 mEq. potassium per L. (volume: 520 ml.).

On this limited evidence it would appear that potassium retention and urinary loss of sodium are features of the onset of an attack.

Response to Sodium Chloride. The use of sodium chloride was suggested by the history of high salt intake among many members of the family and because sodium chloride precipitates classic periodic paralysis which in several respects is a reverse disorder to the one described here. A therapeutic response to the administration of sodium chloride was demonstrated in five separate instances.

One patient (Case 1), who had shown no improvement whatever in his clinical condition after six days of complete paralysis demonstrated marked improvement after being given sodium chloride orally. The patient's sodium balance over six days had been: intake 252 mEq., output 407 mEq., balance −155 mEq. An initial oral dose of 10 gm. of sodium chloride (171 mEq. sodium) had no effect. This amount approximately balanced the sodium loss. However, within an hour of a second dose of 5 gm. of sodium chloride (86 mEq.) which brought the total intake to 509 mEq., cumulative balance to +102 mEq., the patient began to show marked improvement and within five hours was able to walk. Previously he had been unable to sit up in bed and his lower extremities were completely paralyzed. The degree of improvement levelled off, however, well below normal muscle strength. The patient walked with a pronounced limp, and with considerable effort. The next morning, because of persisting weakness, he was given 80 mEq. of sodium chloride intravenously, with further striking improvement in clinical condition. By the time the drip had been completed there was return to normal strength without evidence of limp or disability.

On a second occasion when weakness occurred in this patient (Case 1) intravenous normal saline solution (160 mEq. sodium) was again employed and improved muscle strength by 50 per cent although it failed to correct the weakness completely.

An attack was precipitated by orally administered potassium chloride in one patient (Case 3) who awoke just able to walk, with a considerable limp and much fatigue. After the administration of 1 L. of triple saline solution (462 mEq. sodium) he was able to leave the hospital four hours later without any perceptible impairment of muscular function, although he thought that his muscles still fatigued more easily than was normal.

On a second occasion, after paralysis had been induced by alcoholic excess, the patient had shown no change in his clinical condition for twenty-four hours and had quadriparesis with complete inability to move the legs and inability to sit up in bed. Arm strength was limited to 12 pound pull on a spring scale. He was then given 1 L. triple saline solution (462 mEq. sodium). Two hours after the administration of saline was begun he was able to pull 30 pounds on the scale and had a considerable degree of return of function in the legs. Improvement was then progressive and by the following morning,

TABLE IV
SODIUM BALANCE DURING AN ATTACK OF PARALYSIS IN PATIENT 1 (ATTACK 3)

Period (hr.)	Intake (mEq.)	Output (mEq.)	Balance (mEq.)	Cumulative Balance (mEq.)	Treatment	Extent of Paralysis (0 to +++)
On admission						+++
0–19	800	527	+273	+273	Intravenously administered saline solution	+++
19–45	103	170	−67	+206	Acetazolamide	+++
45–69	0	135	−135	+71	Various drugs	++
69–96	0	45	−45	+26	Nil	++
96–108	50	49	+1	+27	Nil	+

twelve hours later, he could pull 44 pounds and walk about. Normally an episode of paralysis with complete loss of motion in the lower extremities lasts four to seven days in this patient.

One attack in a patient (Case 1) was not significantly affected by sodium chloride therapy. A severe attack of quadriparesis followed withdrawal of 9-alpha-fluorohydrocortisone. The patient was admitted as the attack reached its maximum severity. Triple normal saline solution was administered intravenously that evening (1 L.) and the following morning (750 ml.), without appreciable change in the weakness. Sodium balance figures for the whole admission are shown in Table IV. Several other therapeutic measures were tried, including potassium depletion by means of diuresis induced by the administration of acetazolamide (sodium losses being replaced approximately), but the attack followed its natural course with slow recovery over one week and residual weakness for five subsequent weeks. During the first four days of the attack the patient refused all oral intake except watery fluids.

The changes in plasma electrolytes following sodium chloride administration are summarized in Table V. There was no consistent change in plasma electrolytes to explain the success of this therapy on some occasions. Presumably the beneficial effects of saline solution are due to shifts in fluid and electrolytes rather than changes in concentration.

Our observations to date confirm the long-standing traditions of the family that salt taken at the onset of an attack will reduce its severity or shorten it, but it appears that severe attacks when fully developed may no longer be responsive to this form of therapy, even when large amounts of sodium are retained.

Response to 9-Alpha-Fluorohydrocortisone. The trial of a drug which causes renal retention of sodium and chloride was suggested by the encouraging results with salt during the attacks, and by the consistent high salt intake of several of the patients. Two patients (Cases 1 and 2) were treated with 9-alpha-fluorohydrocortisone, 0.1 mg. daily. Both did well for a period of three weeks without any evidence of weakness despite efforts to precipitate attacks by overexertion followed by complete rest, sleeping late on Sundays, and sitting quietly for several hours at a time. Administration of the drug was then stopped to study the effects of withdrawal. Within twenty-four hours stiffness and weakness developed in both patients. These symptoms could not be reversed twenty-four hours later by the administration of 0.2 mg. 9-alpha-fluorohydrocortisone and increased salt intake, despite the fact that in one patient (Case 1) oedema

TABLE V
PLASMA ELECTROLYTE CHANGES FOLLOWING SODIUM CHLORIDE ADMINISTRATION

Electrolyte	Mean of Three Infusions (Patients 1, 3) Followed by Improvement		One Occasion (two infusions, Patient 1) When No Improvement Occurred	
	Before	After	Before	After
Sodium (mEq./L.)	143	145	140	147
Potassium (mEq./L.)	3.9	3.9	4.5	4.0
Chloride (mEq./L.)	101	103	103	115
HCO_3 (mEq./L.)	27	24	23	22

developed after four days of this intensive therapy. The other patient (Case 2) was able to ward off an early attack on another occasion by increasing salt intake and starting 9-alpha-fluorohydrocortisone therapy; this may have been fortuitous, but the reversal of the attack was more striking than any he had experienced with salt treatment alone.

Other Metabolic Studies. During the course of a severe attack in one patient (Case 1) total exchangeable sodium was estimated by the injection of sodium 24. A level of 36.1 mEq. per kg. was obtained (normal 38.5 ± 2.9) [*12*]. The estimation was carried out on day 4 of the study recorded in Table IV, by which time the disturbance of sodium balance produced by therapy had been corrected; it therefore represents the body sodium level at the height of an untreated attack in this patient.

Response to Other Medications. Attempts were made to alter sodium and potassium balance by the administration of chlorothiazide or acetazolamide during the course of two attacks. The diuretic response was surprisingly poor; a slight negative balance of both sodium and potassium was produced on both occasions without effect on paralysis.

Spironolactone, which had proved successful in the treatment of a case of classic periodic paralysis [*9*], was administered during one attack. A sodium balance of minus 50 mEq. and potassium balance of plus 15 mEq. per twenty-four hours were produced without effect on the course of the attack.

On the evidence presented in the preceding sections the most logical therapy would be one that encouraged potassium excretion and sodium retention between attacks or at the onset of an attack. The combination of acetazolamide and 9-alpha-fluorohydrocortisone has this effect and has been used successfully in one patient (Case 1). He takes 250 mg. acetazolamide and 0.1 mg. 9-alpha-fluorohydrocortisone daily and has not had a single attack of weakness during three months of this therapy, despite all attempts at provocation. Previously he had major attacks of paralysis at least once a month and often had weakness of the legs between major bouts. Acetazolamide therapy alone in the same patient did not correct the weakness between attacks.

Intravenously administered calcium gluconate, which is helpful in attacks of adynamia episodica hereditaria [*3*], was ineffective in one of our patients.

Intravenous injection of the quick-acting cholinergic drug Tensilon® had no effect on muscle power during a severe attack in one patient (Case 1), and intra-arterial injection of acetylcholine into a paralyzed limb of the same patient caused no muscle twitch or increase in power, but was accompanied by flushing and discomfort.

SPECIAL STUDIES OF MUSCLE

Electromyography. Investigation of both weakened and paralyzed muscles in a patient (Case 1) during two episodes of severe paralysis with sensory impairment was carried out with concentric needle electrodes.* Insertion activity was not increased. Numerous short duration polyphasic potentials were found. In moderately weakened muscles the interference pattern was full but it was greatly reduced in severely paralyzed muscles. Several large, long duration potentials with two or three phases were recorded on volition from each muscle. The amplitude of all potentials recorded was greatly reduced.

The excess of myopathic units and full interference pattern despite considerable weakness favours disease of muscle. The large motor units, however, imply an associated neurogenic lesion. Conduction velocity determinations carried out in the ulnar and lateral popliteal nerves were normal.

It is of interest to compare these findings with those reported in classic periodic paralysis in which there is progressive prolongation of action potentials and reduction in their amplitude as paralysis progresses. In adynamia episodica hereditaria action potentials are always significantly shortened during paresis. In addition there are signs of hyperexcitability characterized by numerous spontaneous discharges in resting muscle and increased mechanical excitability. These findings were interpreted as indicating a reduction in the number of fibres which can be activated and increased excitability in the remaining fibres due to partial depolarization of the cell membrane [*13*].

Although the electromyographic findings reported here resemble those of adynamia episodica hereditaria, no evidence of the hyperexcitability of muscle fibres reported in that disease was found.

Muscle Biopsy. Portions of a muscle biopsy specimen obtained at the height of an episode of paralysis were processed in two separate

* By Dr. Alan Ridley, Research Fellow in Neurology.

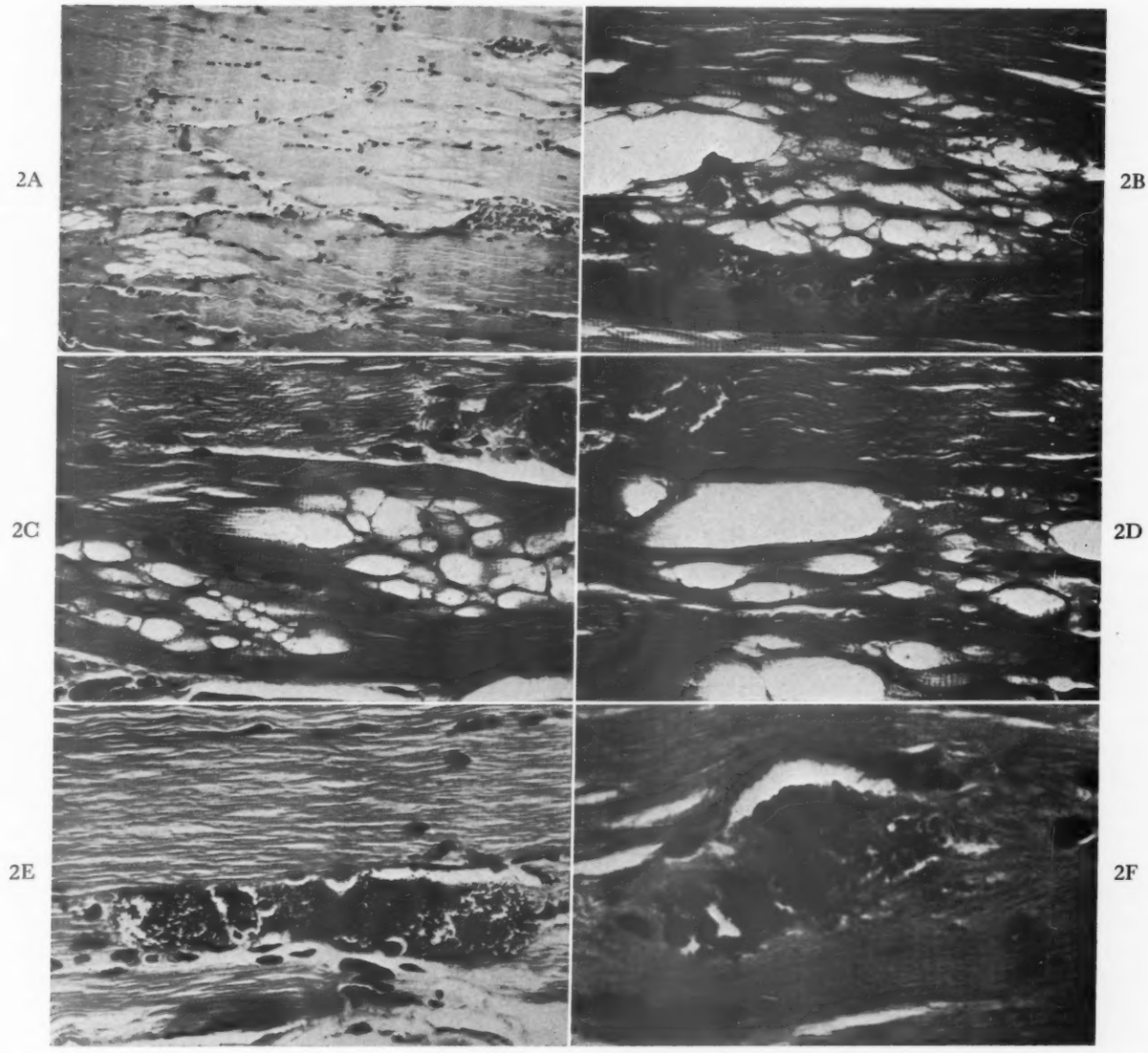

FIG. 2. Sartorius muscle biopsy specimen obtained during a severe attack of sodium responsive, normokalemic periodic paralysis. A, vacuolization and focal areas of cellular reaction. Original magnification × 160. B, C and D, vacuolization of muscle fibers with focal necrosis and cellular response. Adjacent myofibrils show normal striations. Original magnification × 640. E and F, focal necrosis with minimal or absent cellular response. These areas are stained by periodic acid-Schiff and Best's carmine stain. Original magnification × 1600 (E), × 640 (F).

laboratories and stained with haemotoxylin and eosin, periodic acid-Schiff with diastase control, and Best's carmine stain. The specimen, interpreted by Dr. Geoffrey W. Pearce, Research Fellow in Neurology and in Pathology, revealed groups of vacuoles scattered throughout the sections. (Fig. 2.) Some of these contained an amorphous eosinophilic substance or a few disrupted myofibrils; others were empty. The vacuolar walls were hyaline and deeply eosinophilic but the immediately adjacent myofibrils were striated and apparently normal. Blocks from both laboratories gave the same results but more vacuoles were found in one specimen than in the other. Vacuolation within individual muscle fibres has been previously reported in classic periodic paralysis [*12*] and in Tyler's family [*6*] but not in adynamia episodica hereditaria [*14*].

Focal areas of necrosis with hyaline change and disruption of myofibrils were present with and without phagocytosis, indicating a significant acute destructive change. Occasionally areas of myofibril loss were replaced by a mass of deep purple granules. Myofibrils adjacent to such areas were relatively normal. Areas were present where the A-bands were relatively swollen, with a tendency to stain more deeply.

Sarcoplasmic basophilia was not seen and there was no apparent increase of sarcoplasm. Nuclei were generally vesicular and many were enlarged with prominent nucleoli; excessively elongated nuclei were frequent and small hyperchromatic nuclei were rare.

Periodic acid-Schiff positive staining was found in small areas which either involved the whole diameter of the fibre or extended under the sarcolemma for a considerable distance. Some staining was also found in connective tissue. Best's carmine stain showed the granular nature of the deposit better than the periodic acid-Schiff stain and had the same distribution. Positive staining granules were occasionally seen within the vacuoles.

The findings on electron microscopy will be reported separately.

Measurement of Muscle Cell Membrane Potential and Intracellular Cations. Dr. Thomas Scratcherd, of the Department of Physiology, kindly undertook to measure the muscle cell membrane potential using microelectrodes [*15,16*] in the single available sartorius muscle biopsy specimen in the three hours immediately following its removal. Although a single series of observations cannot be considered evidence of an abnormal state in the muscle cell membrane, the results indicated that cell membrane potential was less than normal. These preliminary data are subject to further confirmation.

Eighty per cent of a series of twenty-five recorded cell potentials fell between 29 and 50 mv. Only the five highest determinations, 50 to 63 mv. (averaging 57 mv.), were accepted in view of a later examination of the specimen under phase microscopy* which indicated that the sarcolemma had sealed off in only a portion of the fibres. The normal muscle cell membrane potential in man is 70 to 90 mv.

Determination of intracellular cations in the same biopsy specimen revealed a high level of intracellular sodium and a low concentration of intracellular potassium. The potassium determination when substituted in the Nernst equation resulted in a theoretic membrane potential which agreed with the experimentally determined results corrected for the temperature at which the measurements were conducted [*17*].

COMMENTS

The disease which affects the Southwood-Gannon family differs from classic periodic paralysis and adynamia episodica hereditaria (Table 1), and in the present state of ignorance about its nature we employ the descriptive term "sodium responsive, normokalemic periodic paralysis" (SRNPP). The main distinguishing features from the classic disease are its earlier age of onset, precipitation of attacks by underactivity, sensory impairment which accompanies some severe attacks, the maintenance of a normal plasma potassium throughout the attack, the unfavourable response to potassium and the favourable response to sodium.

It differs from adynamia episodica hereditaria in the relative infrequency, severity and duration of attacks, nocturnal onset, precipitation by underactivity, the sensory changes, the normal plasma potassium and the retention of potassium during attacks. Although attacks can be precipitated by the administration of potassium chloride in both diseases, the response is more consistent and is produced by a much smaller dose in adynamia than in SRNPP. Carbohydrate intake, which seems to ward off attacks of adynamia, has no effect on SRNPP.

Metabolic studies to date suggest other features in which SRNPP differs from the previously described diseases. There is no evidence of the sodium retention before attacks described by Conn and Streeten [*12*] in the classic form, and such observations as we have made suggest that there may even be a natriuresis during the onset of the attack. Total exchangeable body sodium in one patient was normal, in contrast to the high figures quoted by Conn and Streeten, and the pattern of intracellular electrolyte composition in one patient with SRNPP was the opposite of that described in classic periodic paralysis [*12*]. The excessive sensitivity to acetylcholine described by Gamstorp [*3*] in adynamia contrasts with a decreased sensitivity in one of our patients.

The electromyographic findings more closely resemble those of adynamia episodica hereditaria than classic periodic paralysis but differ in several important respects from either. There is evidence of a neurogenic as well as a myopathic lesion.

The histologic appearance of muscle tissue, on the other hand, is similar to that reported in classic periodic paralysis. (Tyler's family [*6*] in which vacuolation of muscle fibres was described, more closely resembles the entity described here than adynamia episodica hereditaria with which it has been identified [*18*].)

* By Dr. Geoffrey W. Pearce.

TABLE VI
CLASSIFICATION OF PERIODIC PARALYSIS

Classification	Electrolyte Disturbance
I	Hypokalemic: classic periodic paralysis (a) with preceding sodium retention and excess aldosterone excretion [*11,19*] (response to very low sodium diet, aldosterone inhibitors, potassium chloride) (b) without preceding sodium retention and without excess aldosterone excretion [*20*] (response to potassium chloride)
II	Hyperkalemic: adynamia episodica hereditaria [*3*] (response to acetazolamide prophylactically, calcium in attacks)
III	Normokalemic: normokalemic sodium responsive periodic paralysis (response to 2-methyl-9-alpha-fluorohydrocortisone and sodium chloride)

TABLE VII
THERAPY IN THREE TYPES OF PERIODIC PARALYSIS

Type	Prophylaxis	Treatment of Attack
Classic periodic paralysis	Very low salt diet [*11*] Spironolactone [*9*] Potassium chloride	Potassium chloride
Adynamia episodica hereditaria	Acetazolamide [*21,22*] Chlorothiazide [*21,22*] Dextroamphetamine [*14*] Thyroid Cod liver oil	Calcium gluconate [*3*]
Sodium responsive, normokalemic periodic paralysis	9-alpha-fluorohydrocortisone with acetazolamide High salt intake	Sodium chloride

The dissimilarities listed appear to us adequate grounds for distinguishing sodium responsive, normokalemic periodic paralysis as a separate disease entity, although the three conditions have many features in common. This confusing picture might be clarified if it could be shown that the three diseases were due to three inherited enzymatic defects which affected the same metabolic pathway at different points. It is conceivable that the pathway involved is concerned with muscle carbohydrate metabolism or resynthesis, in view of the response to rest after exercise which is common to all three diseases; some evidence for this view was presented by McArdle [*7*] in a case of classic periodic paralysis, and the occurrence of glycogen-containing vacuoles in the muscle cells in classic periodic paralysis, SRNPP and Tyler's family suggests a defect in carbohydrate metabolism.

It is also possible that paralysis is the result of alteration in the membrane potential produced as a byproduct of excessive electrolyte transfer across the cell wall when the defective enzyme pathway is overloaded, or when sodium or potassium is administered. The single measurement of cell membrane potential recorded here, although less than the normal level, can at best only be considered suggestive until additional observations are available.

The presence of peripheral sensory loss during attacks in one patient and the electromyographic evidence both point to a defect in the neuron or neuronal transmission in addition to the more prominent alteration in muscle activity. These findings also are unexplained. The suggestion of Conn and Streeten that excessive aldosterone secretion underlies all types of periodic paralysis seems to us unlikely in view of the wide variations in potassium and sodium metabolism in the three types. These speculations will be resolved only by more adequate studies of muscle carbohydrate metabolism in the three diseases. It may be that there are several other genetic defects capable of producing a superficially similar clinical picture, and further disintegration of periodic paralysis into different syndromes can be expected.

Until a basic pathologic mechanism becomes apparent, patients with periodic paralysis may be classified in three main groups (Table VI) on the basis of clinical features, plasma potassium levels during the attack and the response to administered potassium chloride and sodium chloride. It would appear that the classic hypokalemic type of periodic paralysis is subdivided into two distinct categories: those cases in which a preceding sodium retention or excess aldosterone excretion can be demonstrated [*11,19*] and at least one study in which neither of these abnormalities was detected [*20*].

In any affected family each disease appears to run true to type in all affected members.

Therapy that has proved helpful in each of the three types is summarized in Table VII. (For fuller details see Conn and Streeten [*12*].) The successful use of acetazolamide and chlorothiazide in adynamia episodica hereditaria is reported by McArdle [*21*] and by Walton [*22*].

SUMMARY

A family with forty-five members is described, twenty-one of whom have suffered from periodic episodes of paralysis. The illness starts in the first decade and is characterized by episodes of paralysis at intervals of one to three months lasting from two days to three weeks, often of a severe degree including quadriplegia and weakness of the muscles of mastication but excluding facial expression, bladder and bowel function, and respiration. Sensory loss to pain and temperature occurred in one patient studied.

The pattern of inheritance is autosomal dominant.

Episodes are provoked by rest after physical exertion, prolonged inactivity such as sleeping late in the morning, or sitting or standing in one place for several hours. Alcoholic intake, cold and damp, and mental stress also predispose to attacks. Administration of potassium chloride brings on an attack or increases the degree of paralysis. The plasma potassium level is normal during attacks. Sodium chloride has been useful in treating attacks of paralysis, and a combination of 9-alpha-fluorohydrocortisone and acetazolamide has proved effective in preventing attacks.

Muscle biopsy specimens obtained during an attack demonstrated vacuolation of muscle fibres and focal areas of muscle fibre degeneration.

The disease has many features in common with classic periodic paralysis and adynamia episodica hereditaria but sufficient dissimilarities occur to justify its description as a third separate syndrome.

Acknowledgment: We should like to express our appreciation to Dr. Henry Miller and Professor George Smart for advice and encouragement; to Dr. John N. Walton for aid in finding many of the patients and for use of his earlier data; to Dr. A. Louis Latner for biochemical determinations; to Dr. Geoffrey W. Pearce for muscle pathology and phase and electron microscopy; to Dr. E. J. Field for microphotography; to Dr. Alan Ridley for electromyography; to Dr. Thomas Scratcherd for determination of muscle cell membrane potentials and intracellular electrolytes; and to Drs. Margaret Irvine and Leo Kinlen for assistance with metabolic studies.

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Relationship of Multiple Endocrine Adenomas to the Syndrome of Ulcerogenic Islet Cell Adenomas (Zollinger-Ellison)*

Occurrence of Both Syndromes in One Family

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THE syndrome of multiple endocrine adenomas (MEA) is defined as the simultaneous occurrence of adenomas of the anterior pituitary, the parathyroids, and the islet cells of the pancreas. The adenomas may be hormonally active in all three glands or in only two of them, in any combination. The syndrome was first observed by pathologists about fifty years ago and on several occasions since [*1–7*]. It has been described by clinicians only recently [*8–11* and others].

The clinical picture is variable, depending mainly upon which glands are involved and whether or not the adenomas are functioning. The first manifestations of the disease are usually produced by hyperfunction of only one endocrine gland. After a variable period of time evidence of hyperfunction appears, rarely of deficiency of the other gland(s), and finally, a complex clinical picture results in which the different metabolic disturbances become hardly recognizable.

Wermer [*10*] described MEA as a hereditary disease caused by a dominant gene. Evidence for familial occurrence of the syndrome has been reported in only two other instances [*11,12*]. In addition, peptic ulcers frequently have been found in patients with MEA [*6,9–11,13–16*]. Gastrointestinal ulcers have also been noted among relatives of patients with MEA [*9,11,14*].

Islet cell adenomas not secreting insulin and of non-β-cell origin may produce an ulcerogenic factor of probably endocrine nature which stimulates gastric secretion. In 1955 Zollinger and Ellison [*17*] recognized as a new disease entity a triad consisting of (1) peptic ulcers which are often atypically located, progressing despite all known medical therapy, radiation and/or surgical procedures; (2) hypersecretion of gastric juice, with values as high as 3,000 ml. and hydrochloric acid output up to 300 mEq. within a twelve-hour period; and (3) adenomas of the pancreatic islet cells consisting of non-β-cells. The reports of seventy-five cases of this syndrome have been published thus far. The hypothetic ulcerogenic hormone has not yet been isolated and its action in experimental animals has not been demonstrated [*16–19*]. The pancreatic tumors are multiple in about 50 per cent of the cases and in several instances β-cell adenomas were present simultaneously, producing symptoms of hyperinsulinism. In addition to the usual pancreatic site, the tumors are occasionally found in the hilus of the spleen or in ectopic pancreatic tissues in the gastric or duodenal mucosa. After removal of the ulcerogenic tumor(s) the patients may be cured. However, total extirpation is often difficult or impossible and therefore Zollinger recommends total gastrectomy as the safest procedure for symptomatic cure.

The two syndromes of MEA and the ulcerogenic islet cell adenoma (UIA) seem to be related in some way. In about one fifth of the reported cases of UIA there was also a diagnosis of MEA [*16–22*]. In about one fifth of the reported cases of MEA there is evidence from the history or the laboratory data for the presence of UIA also.

In 1939 Rossier and Dressler [*14*] described a

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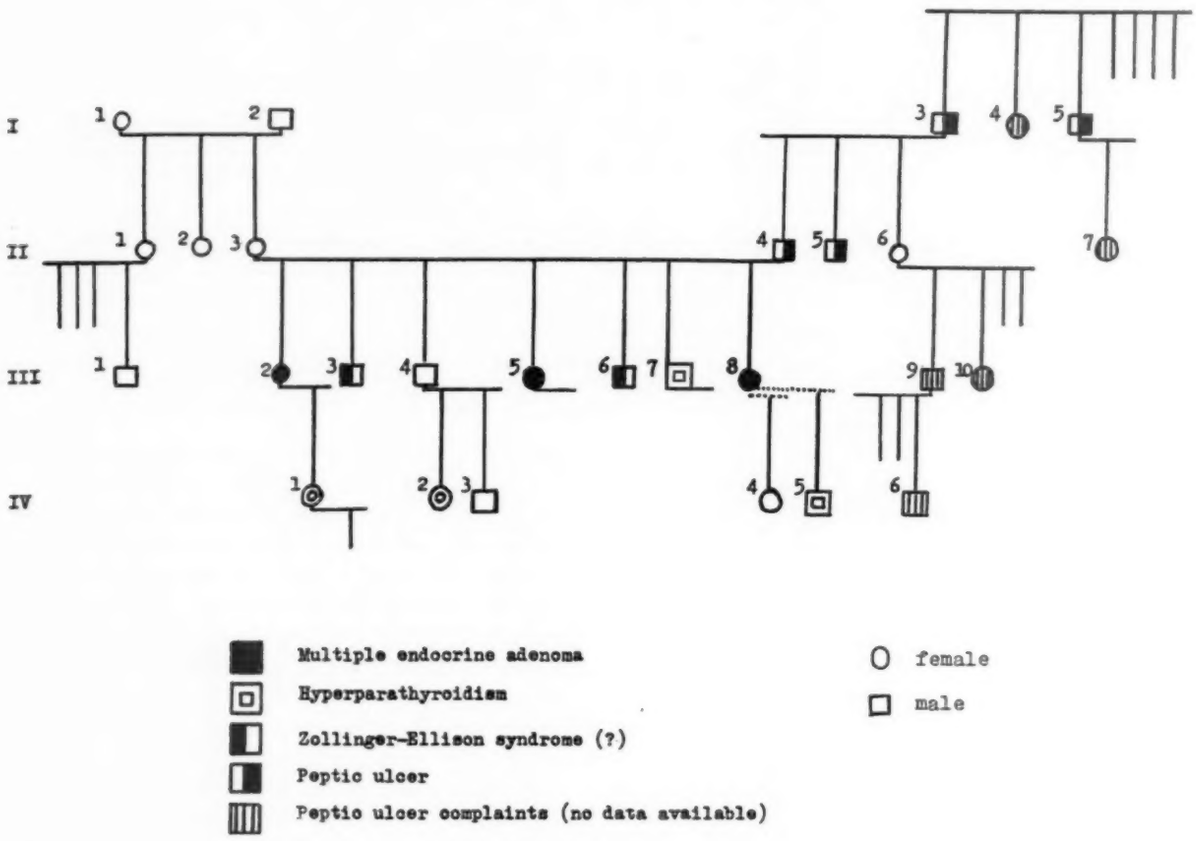

FIG. 1.

family in whom peptic ulcers were frequent; in addition, one member had MEA and another had recurrent Cushing's syndrome. An extended study of this family is presented in the present report: there are now three members with MEA, four with isolated hyperparathyroidism and six with peptic ulcer. In two patients with gastrointestinal ulcer there is strong clinical evidence for the presence of UIA. A detailed presentation of this pedigree will provide the opportunity to draw some conclusions about the mode of inheritance of MEA and the relationship to peptic ulcer.

CASE REPORTS

Of the 200 members of the family, traced back to about 1700, forty-one were investigated further as indicated in the pedigree presented. (Fig. 1.) The ancestors were a family of farmers in the canton of Zurich. Our genetic study of the paternal and maternal forebears carried out over a period of seven generations did not show any evidence of consanguinity.

From twenty-eight members of the family a detailed history could be obtained by personal inquiry or reports of the family physicians. For twelve members we could obtain hospital records or necropsy findings. In respect to four other members we had more general data about their histories and physical condition from various sources and were able to get more specific information by determining the calcium, inorganic phosphorus and alkaline phosphatase levels in the serum.

Patients with Multiple Endocrine Adenomas

CASE III/2. A forty-three year old married white woman (Z. G.), born in 1903, was first seen at the Neurosurgical Hospital of Zurich on April 24, 1946, complaining of headaches and progressive visual disturbances of one year's duration.

Her childhood had been uneventful. Menarche began at age nineteen. She delivered a normal infant following a normal pregnancy in 1937. There was premature menopause in 1940.

The physical examination disclosed bitemporal hemianopsia, partial optic atrophy and para-axial scotoma. Marked sellar enlargement was noted on the roentgenograms of the skull. Results of the oral

glucose tolerance test indicated an insufficient rise in the blood sugar. On May 8, 1946, an encapsulated chromophobe pituitary adenoma, the size of a walnut, was removed surgically. During the operation the pituitary was noted to be flattened underneath the tumor, which occupied the pituitary fossa.

A few days after her discharge from the hospital the patient fell into a state of agitation, excitation and mental confusion. She was hospitalized again on May 25, 1946, in a semi-comatose state. Hypoglycemia of 30 mg. per 100 ml. was found, and only with massive infusions of glucose could this condition be relieved. Among other studies carried out on the patient were normal acidity of the unstimulated gastric juice, flat glucose tolerance curve with hypoglycemic values and no response to adrenalin, and normal basal metabolic rate of plus 12 per cent. The serum calcium was 11.5 mg. per 100 ml., the alkaline phosphatase 8 Bodansky units. Other electrolyte studies gave results within normal limits.

One month later the patient had to be admitted for the third time as an emergency case. As before, marked hypoglycemia was the cause of the coma; the blood sugar was found to be 20 mg. per 100 ml. at this time. Almost daily infusions of glucose were required to control the hypoglycemic spells which occurred regularly a few hours after the discontinuation of the intravenous glucose drip. Usually, the blood sugar fell to levels of 10 or 20 mg. per 100 ml.; on several occasions it was found to be practically zero. It was not possible to keep this severe hypoglycemia permanently under control and the patient subsequently died from her ailment on September 27, 1947, after several temporary remissions. She was forty-four years of age at the time of death.

The clinical diagnoses were chromophobe adenoma of the pituitary; hyperinsulinism; hyperparathyroidism (?).

At necropsy a pituitary body remnant of 10 by 2 mm., with intact histologic structure, was found. The pancreas was grossly normal. On microscopic examination numerous islets were noted in all the sections made through this organ. There was one microscopic islet cell adenoma. A colloidal nodular goiter was found with one atypical area which proved to be a malignant goiter (Langhans). A solitary metastasis was seen in the spleen. The three layers of the adrenals could be well distinguished, yet presented several small hemorrhagic areas.

Comment: The patient was a white woman who had an early menopause at age thirty-seven; eye symptoms developed at age forty-two. One year later a chromophobe pituitary adenoma was removed. One month postoperatively there were severe hypoglycemic attacks which could not be brought under control for any length of time and led to the patient's death at the age of forty-four. Necropsy studies revealed an intact remnant of the body of the pituitary and diffuse adenomatosis of the pancreatic islet cells, with a microscopic islet cell adenoma.

CASE III/5. A twenty-five year old white married woman (B. H.), born in 1907, was seen by her local physician in April 1932 because of menstrual irregularities. The periods occurred only every six to twelve weeks. To a lesser degree there had been some irregularity of the menstrual cycle since the patient's eighteenth birthday. At that time, there was a temporary amenorrhea of about one year's duration. The past history was otherwise essentially non-contributory. The menarche had been normal.

In June 1932 this woman had cramps of the extremities for the first time, and convulsions with loss of consciousness. After each fit she would sleep for one to one and a half hours. These spells most frequently occurred at night or in the morning before breakfast. The patient had a recent weight gain of 35 pounds.

In May 1933 she had to be admitted to the Hospital for Epileptic Disorders. In several of the observed seizures there was loss of consciousness and tongue biting. On one occasion an intense tremor of the left hand was noted. At this time the patient's weight was 180 pounds. She had rather masculine facial features whereas the body hair was of feminine distribution. The blood pressure was measured at 120/70 mm. Hg. Examination of the eyes (including perimetry), neurological examination, spinal fluid examination and routine blood tests were all within normal limits. The serum calcium at the time of admission was 11.2 mg. per 100 ml.

The patient was readmitted to the hospital on February 7, 1934, because of recurrent seizures. Despite anticonvulsive medication, seizures continued to occur intermittently at night. There was shaking of the body, incontinence, perspiration, tongue biting, and the patient progressively became more drowsy and somnolent. She had a flushed face, a tachycardia of 130 per minute, and a deep, noisy, regular respiration. On neurologic examination some slight degree of asymmetry was noted in muscle tone and tendon reflexes, yet there were normal findings in the spinal fluid. The patient was subsequently found to have fever with temperatures as high as 105°F., cardiac arrhythmia with a rate varying between 32 and 160 per minute, Cheyne-Stokes respiration, and myoclonic shaking. She died on February 21, 1934.

At necropsy (carried out at the Institute for Pathology of Zurich) multiple endocrine adenomas were found. A parathyroid adenoma the size of a bean was noted and there were several rough concretions in the renal pelvis bilaterally. The largest of these calculi measured 3.5 by 1.5 by 1 cm. There was an adenoma of the pancreas, probably of islet cell origin. An enlarged parapancreatic lymph node contained a grayish, homogeneous tumor mass which was made up

of polygonal and spindle-shaped cells arranged around capillaries. No mitoses were noted. Fibers of connective tissue divided this tumor mass into small lobules, and in the marginal zone several pancreatic ducts were found. The adrenals presented minimal evidence of fibrosis, but the pituitary and thyroid glands were found to be intact.

From the clinical and autopsy data available it became evident that the patient's spells had been caused by hypoglycemia. This hypothesis was further confirmed by several determinations of the blood sugar, performed a few days antemortem. A relationship of the patient's psychic state to the degree of hypoglycemia was evident. With a blood sugar level of 61 mg. per 100 ml. the patient was mentally clear and quiet; with a level of 41 to 59 mg. per 100 ml. she did not respond readily and presented a variable degree of cramping of the extremities. When the blood sugar level reached 30 mg. per 100 ml. the patient was described as soporific and inclined to have seizures.

From the autopsy report it cannot be decided whether the hyperinsulinism had been caused by an adenoma or a carcinoma of the islet cells. However, there is evidence to assume that the parapancreatic lymph node, which was found to be invaded by a tumor mass, represented a metastasis of a functioning islet cell carcinoma.

The final diagnoses were hyperinsulinism and hyperparathyroidism; islet cell adenoma or carcinoma; adenoma of the parathyroid glands.

Comment: In retrospect, this twenty-five year old woman suffered from hypoglycemic spells. There was, in addition, evidence of simultaneous hypercalcemia. The patient died at age twenty-seven in hypoglycemic coma. At necropsy an adenoma of the parathyroids was noted and an islet cell adenoma of the pancreas, probably an adenocarcinoma with regional metastases, was found.

Case III/8. A forty-four year old white married woman (K. A.), born in 1916, was admitted to the hospital for the first time in 1935 (at the age of nineteen years) because of right-sided abdominal pain. Her past history was essentially non-contributory. Menarche had occurred at age fourteen, and the menstrual periods had been regular.

An excretory urogram at the time of admission revealed bilateral hydronephrosis. Because of persistent colicky pain, an exploratory operation was performed on the right kidney, but no calculus could be found. In 1937 this patient experienced similar pains on the left side and on that occasion a spindle-shaped calculus 1 cm. in length was removed from the lower left ureter. Roentgenologically, several calculi were noted in the right renal pelvis.

On October 19, 1938, the patient was hospitalized again for further studies because she had had a recent weight gain of 45 pounds and amenorrhea of six months' duration. The diagnosis of Cushing's syndrome was made. On admission, the patient presented a plethoric appearance, obesity which was confined to the face and trunk, and purplish striae on the lower part of the abdomen. The blood pressure was 150 mm. Hg systolic and 110 mm. Hg diastolic. The fasting blood sugar was 141 mg. per 100 ml. and a constant slight glycosuria was present. The result of the oral glucose tolerance test further confirmed the diagnosis of diabetes mellitus. X-ray studies revealed a normal sella turcica and a minor degree of osteoporosis in the thoracic and lumbar spine as well as in the pelvis. The serum calcium values were 10.8, 12.1 and 12.4 mg. per 100 ml. on three separate determinations. The serum phosphorus was 3.4 mg. per 100 ml. and calcium excretion in the urine (after a Snapper diet) was 175 mg. per twenty-four hours.

Since the diagnosis of Cushing's syndrome was confirmed, the patient was treated with x-ray to the pituitary. A total dose of 6,960 r was given. After four months the periods returned, the blood pressure and blood sugar reverted to normal levels, and there was a gradual reduction in weight. In general, the patient did quite well.

In May 1945 a pyelotomy was performed because of recurrent episodes of renal colic, and an oval-shaped calculus was removed from the right renal pelvis.

In November 1946 there was a recurrence of Cushing's syndrome, with a weight gain of 35 pounds, amenorrhea, polydipsia and general fatigue. The fasting blood sugar was 260 mg. per 100 ml. with a glycosuria of 6 per cent. The basal metabolic rate was normal. The blood pressure was measured at 190 mm. Hg systolic and 110 mm. Hg diastolic, and a slight polycythemia of 5.6 million red cells was noted. The neurologic examination (including funduscopy and perimetry) was within normal limits.

A second course of roentgen therapy to the pituitary was carried out (9,000 r), again with a good response. After a few months the menstrual cycle became normal, the blood pressure dropped to 110 mm. Hg systolic and 80 mm. Hg diastolic, and there was a weight loss of 48 pounds.

In the following years the patient was periodically checked at the clinic. She has never presented any evidence of recurrence of Cushing's syndrome, since the menses, blood pressure and blood sugar were always found to be normal. However, the serum calcium was usually found to be somewhat high. On May 13, 1947, the serum calcium was 11.3 mg. per 100 ml., the inorganic phosphorus 2.1 mg. per 100 ml. and the alkaline phosphatase 5.1 Bodansky units. On August 16, 1949, the calcium was 11.1 mg. per 100 ml. and the phosphorus 2.7 mg. per 100 ml.

In 1948 the patient delivered a female child after a normal pregnancy, and one and a half year's later a son was born. Both children were healthy and their development was normal.

The patient had practically no symptoms over a period of about ten years. In 1956, however, she frequently felt fatigued and weak, complained of headaches, backaches, occasional dizziness, and gave a history of excessive thirst and polyuria. On admission a serum calcium level ranging between 11.5 and 12.4 mg. per 100 ml. was found, and a phosphorus level as low as 1.7 mg. per 100 ml. The serum alkaline phosphatase was normal or only slightly increased. The calcium excretion in the urine (after a Snapper diet) varied between 140 and 280 mg. per twenty-four hours. Based on these data hyperparathyroidism was diagnosed. A bone biopsy specimen was obtained but was not conclusive: small osteoid zones were noted and also fibro-osteoblastic changes. Other causes for hypercalcemia, such as malignancy, vitamin D intoxication or sarcoidosis, could be excluded.

In January 1959 the patient was hospitalized again because of recurrence of fatigue, weakness, dizziness and polydipsia. The periods were regular. Laboratory studies revealed a constant hypercalcemia (11.6 to 12.3 mg. per 100 ml.) and hypophosphatemia (1.6 to 1.8 mg. per 100 ml.), a serum total protein of 6.5 gm. per 100 ml. and a calcium excretion in the urine of 382.7 mg. per twenty-four hours. A slight decrease in phosphate clearance, with a reabsorption of 76 per cent was noted (normal value: 93 per cent). To exclude a functioning islet cell adenoma, fasting blood sugar determinations over a period of twenty-four hours were obtained. The blood sugar dropped to a minimal level of 72 mg. per 100 ml., but the patient felt well. Results of a glucose tolerance test, urinary steroid studies, and tests for thyroid function were all within normal limits.

Normal findings on roentgenograms of the skeleton and the kidneys contributed to the decision to delay parathyroid surgery. With the exception of small phalangeal cysts, no changes typical of hyperparathyroidism were noted. A slight degree of osteoporosis of the alveolar ridge was present, but there was no atrophy of the lamina dura. There was no evidence of nephrocalcinosis on x-ray examination, and results of renal function studies (which included phenolsulfonphthalein dye excretion test and inulin clearance) were within normal limits.

Hyperparathyroidism, present in this patient for many years (possibly for more than twenty-five years), had not produced demonstrable damage to the organ systems usually involved. For this reason it was decided that surgery should not be performed on this patient at that time. From the results of follow-up examination in January 1959 it was concluded that a particularly mild, non-progressive, chronic form of hyperparathyroidism was present.

The clinical diagnoses were recurrent Cushing's syndrome (with episodes in 1938 and 1947); hyperparathyroidism.

Comment: A nineteen year old woman suffered with recurrent nephrolithiasis. At the age of twenty-two years hypercalcemia was found. At the same time Cushing's syndrome developed, but remission followed roentgen therapy of the pituitary. The patient had two normal pregnancies thereafter. In 1956 hyperparathyroidism was diagnosed, despite the absence of manifestations in the kidneys and the skeleton. In 1959 a recheck did not give any evidence for progression of the hyperparathyroidism and it was decided to delay the operation for this reason.

Patients with Hyperparathyroidism, without Clinical Symptoms of Pituitary or Pancreatic Involvement

Several cases of hyperparathyroidism were detected among relatives by determining the calcium, inorganic phosphorus and alkaline phosphatase levels in the serum, but extensive laboratory examinations (including blood sugar determinations and pituitary function studies) could be obtained in only one (Case III/7).

Case III/7. A thirty-nine year old white man (K. H.), the brother of the three women just described, was hospitalized in 1952 because of episodes of renal colic. A calculus the size of an almond was removed surgically from the right renal pelvis. The past history of this patient had been uneventful.

In subsequent years the patient complained of easy fatigue, weakness, and lack of energy and initiative which he had not noticed before the operation.

On April 15, 1959, the patient was readmitted to the hospital because of headaches and diplopia of several weeks' duration. He was found to have horizontal nystagmus, papillary edema of the right eye of 2 to 3 diopters, and partial paralysis of the right abducens nerve. Carotid angiography suggested a frontotemporal tumor. On May 1, 1959, a meningioma, about the size of a hen's egg, was removed from the lateral part of the right frontal lobe.

Further examination disclosed a hypercalcemia of 12.3 to 13 mg. per 100 ml., and values for inorganic phosphorus of 2 to 2.3 mg. per 100 ml. The serum alkaline phosphatase ranged between 5.9 and 6.4 Bodansky units. The calcium excretion in the urine (after a Snapper diet) was found to be 492 mg. per twenty-four hours. The excretory urogram revealed large concretions and a slight hydronephrosis on the left side. There were also several small calculi in the right renal pelvis. There was a total phenolsulfonphthalein dye excretion of 53 per cent within 120 minutes. The results of a glucose tolerance test and the basal metabolic rate were within normal

limits. The 17-ketosteroid excretion in the urine was slightly increased (39.6 mg. and 24.5 mg. per twenty-four hours) as well as the excretion of 17-hydroxycorticoids (18.5 and 21.8 mg. per twenty-four hours).

On June 6, 1959 surgical exploration of the parathyroids was undertaken and a tumor, almost the size of a walnut, was found near the trachea, below the inferior thyroidal artery. This rather soft tumor had a smooth, violet brown surface. No direct relationship to the thyroid gland was noted, since the tumor was completely embedded in fat tissue. Histologic examination revealed a parathyroid adenoma with cystic transformation.

The expected postoperative drop in the calcium level failed to occur. Initially, the serum calcium level was found to be 11.7 mg. per 100 ml., and it increased to 12.9 mg. per 100 ml. one week later. One month after extirpation of the adenoma serum calcium values ranging from 12.6 to 13.6 mg. per 100 ml. were obtained. From this postoperative course it must be assumed that multiple parathyroid adenomas were present in this patient.

The final diagnoses were hyperparathyroidism; meningioma.

CASE IV/5. A nine year old white boy (K. P.), born in 1950, was described by the family doctor as a healthy, bright child. In screening family members he was found to have a serum calcium of 10.5 mg. per 100 ml., inorganic phosphorus of 1.5 mg. per 100 ml. and serum alkaline phosphatase of 12.5 Bodansky units. Since this boy had a well developed skeletal structure, it was unlikely that he suffered from rickets, and it must be assumed that the low phosphorus value was due to parathyroid hyperfunction.

CASE IV/1. A twenty-four year old white woman (B. E.), born in 1936, was in good health according to her family physician. However, she was found to have a hypercalcemia of 12.1 mg. per 100 ml., and an inorganic phosphorus of 2.3 mg. per 100 ml. The serum alkaline phosphatase level was normal.

CASE IV/2. A twenty-two year old white woman (K. S.), was born in 1938. Her father died at the age of forty-one from a mediastinal sarcoma. She was found to have a slight hypercalcemia of 11.3 mg. per 100 ml. and an inorganic phosphorus of 2.7 mg. per 100 ml. The serum alkaline phosphatase level was normal.

Patients with Peptic Ulcers of the Gastrointestinal Tract

Ulcers of the gastrointestinal tract were evident on roentgenogram or at necropsy in six of the forty-one members of the family (Fig. 1); all six patients died from this ailment before reaching the age of forty-two. In five additional members, there was a history of ulcer complaints of several years' duration. Objective data, however, were not readily available. In this family peptic ulcers occurred almost exclusively in males. In two instances the clinical picture and the course of the disease included features not commonly seen in peptic ulcer disease. In one instance there is strong evidence for a Zollinger-Ellison syndrome, and in a second the presence of this syndrome is possible. In the remaining four cases the data are insufficient to classify the type of peptic ulcer.

CASE III/6. A twenty-five year old white man (K. F.), born in 1908, was seen at the hospital for the first time in 1933 because of severe upper abdominal pain, occurring while fasting and only temporarily relieved by the intake of food. There was no evidence of peptic ulcer on x-ray examination. The only abnormal laboratory finding was gastric juice which contained 130 (normally less than 70) mEq. and 110 (normally less than 55) mEq. of total acidity and free hydrochloric acid, respectively.

In June 1936 the patient was readmitted to the hospital as an emergency case because of recurrence of ulcer symptoms. On laparotomy, yellowish green exudate was present in the abdominal cavity. The entire stomach, however, was of grossly normal appearance. Further examination of the intestinal tract disclosed, in the first portion of the jejunum, a round perforation of 0.5 cm. in diameter with sharp edges and an elevated border. The gross appearance was typical of a hypertrophic peptic ulcer. The patient died shortly after the operation.

At necropsy the stomach was intact. In the second part of the duodenum an ulcer, 1 cm. in diameter, was found. There were multiple ulcers, 0.5 to 1 cm. in size, in the first portion of the jejunum. One of these ulcers, 50 cm. beyond the duodenojejunal flexure, was almost perforated. The ulcer which had been closed surgically was located in the upper jejunum, 5 cm. from the duodenojejunal flexure.

It was not possible to examine the pancreas and duodenum histologically because there was a high degree of putrefaction in these organs when they were seen in the Institute for Pathology. However, it was still possible to identify ectopic pancreatic tissue within the duodenal mucosa. There were areas in which Brunner's glands were replaced by glandular tissue of the pancreas.

The final diagnoses were multiple, perforating ulcers of the first portion of the jejunum and midportion of the duodenum; ectopic pancreatic tissue in the duodenum; Zollinger-Ellison syndrome (?).

Comment: A twenty-five year old brother of the three sisters with MEA, suffered from ulcer symptoms and was treated medically for a period of three years. Hyperacidity and hypersecretion of the gastric juice were noted, although no ulceration was demonstrated roentgenologically.

Necropsy revealed multiple ulcers of the upper jejunum, one ulcer in the second part of the duodenum, and ectopic pancreatic tissue within the duodenal mucosa. The pancreas itself could not be examined because of putrefaction.

CASE III/3. A twenty-seven year old white man (K. K.), born in 1905, was hospitalized in January 1932 because of ulcer complaints of one year's duration, which had resisted medical treatment. Roentgenograms disclosed a duodenal ulcer. The ulcer failed to respond to further attempts at medical management and surgery became necessary. At operation an ulcer was present in the first part of the duodenum and marked fixation, by adhesions, of this part of the stomach to the pancreas was noted. A Billroth II type resection was carried out with anterior gastroenterostomy and Braun's enteroanastomosis, following which the patient showed improvement. However, a few months later severe abdominal pain recurred, and in March 1933 another operation had to be carried out. At the site of the previous anastomosis a closed perforation pointing toward the anterior abdominal wall was found. In addition, there was another perforation of the transverse colon, with abscess formation. Despite an attempt to close the perforation, the patient died in the postoperative period. Histologic examination of the organs was not performed.

CASE II/4. This patient (K. O.), born in 1874, was the father of the seven siblings herein described. He suffered for about six years from an ulcer of the stomach and was hospitalized on several occasions. According to the available hospital records surgery was performed in 1916 for a stomach ulcer and he subsequently died in the same year, at the age of forty-two.

CASE II/5. K. E., the father's brother, was born in 1877. He died suddenly in 1919 at the age of forty-two of a stomach ulcer.

CASE I/3. K. K., born in 1848, was the grandfather of the seven siblings. He died at the age of forty of a stomach ulcer from which he had suffered for several years.

CASE I/5. K. A., born in 1861, was the grandfather's brother. He suffered from upper abdominal pain for many years and was admitted to a small county hospital in 1914 for medical treatment. While there a septic fever developed and he died within a few days. At necropsy a contracted stomach was found, with a markedly thickened wall. No pathologic diagnosis, however, was reported.

During investigation of the family tree, several members were noted to suffer or to have suffered from stomach trouble (pain, burning sensation relieved by food intake, etc.). The exact etiology of these complaints could not be elucidated. Cases I/4, II/7, III/9, III/10, and IV/6 are included in this group.

OTHER TUMORS AND DISTURBANCES OF METABOLISM

Obesity was present in Cases I/1, I/2, II/1, II/2, II/3, III/1, III/4, III/5, III/8 and IV/3.

Diabetes mellitus: The mother of the seven siblings (Case II/3) suffers from mild diabetes mellitus. She is eighty-three years of age.

Goiters: Adenomatous goiters were found in Cases II/2 and II/3. However, because of a high incidence of goiter in Switzerland, this finding is not considered to be of significance. In Case III/2, a malignant goiter (Langhans) was found at necropsy.

Malignancies: In addition to Case III/2 (malignant goiter), in Case III/4 death occurred at the age of forty-one of a mediastinal sarcoma.

Benign tumors: Meningioma was present in Case III/7.

No symptoms of organic disease were noted in two of the descendents of the seven siblings. These two patients (Cases IV/3 and IV/4) had no gastrointestinal complaints and the serum levels of calcium and phosphorus were normal.

Summary of the Case Reports. Three of the seven siblings of this family suffered from multiple endocrine adenomas, one had multiple parathyroid adenomas. Two died of atypical gastrointestinal ulcers which had been resistant to medical and surgical treatment. In one instance a Zollinger-Ellison syndrome was present in all likelihood, and in another instance possibly present. Three of the five descendents of these siblings have hypercalcemia, suggesting a relationship between this symptom and the endocrine disorders (mainly MEA) of the close relatives.

Among the paternal relatives and their descendents six deaths from ulcers are known to have occurred, and five members were found to have stomach complaints which had not been further evaluated.

COMMENTS

The present study is primarily concerned with a family of seven siblings, in which three sisters had multiple endocrine adenomas and one brother suffers from hyperparathyroidism. Two other brothers died of recurrent, intractable peptic ulcers. It is possible in both instances, and very likely in one, that ulcerogenic islet cell

TABLE I
FREQUENCY OF COMBINATIONS OF ADENOMAS IN FORTY-ONE CASES REPORTED IN THE LITERATURE (INCLUDING OUR THREE CASES)

Combination	Cases	
	No.	%
Pituitary, parathyroids and islet cells	14	34
Pituitary and parathyroids	10	25
Pituitary and islet cells	8	19
Parathyroids and islet cells	9	22

TABLE II
INCIDENCE OF ADENOMAS IN ANY ONE ENDOCRINE GLAND IN THE FORTY-ONE REPORTED CASES (INCLUDING OUR CASES)

Location	Cases	
	No.	%
Pituitary	29	71
Parathyroids	32	78
Islet cells	18	44

adenoma was the cause of these early deaths. The only brother in whom neither one of the two syndromes was suspected died of a mediastinal sarcoma. However, there is evidence that he was a carrier of the disease, transmitting it to his daughter who presents the chemical blood changes of hyperparathyroidism.

The ulcer diathesis can be traced over two generations among the paternal ancestors. In fact, a majority of the members in these two generations give evidence of peptic ulcer. Unfortunately, it is not possible to decide from the data available whether the ulcers were of the commonly encountered type or of an unusual type (UIA).

Among the descendents of the seven siblings, five children were studied and in three of them evidence of hyperparathyroidism was found.

Of the three sisters with MEA, one has Cushing's syndrome (basophilic pituitary adenoma ?) combined with hyperparathyroidism. In another case a chromophobe pituitary adenoma was associated with islet cell adenoma, and in the third case islet cell and parathyroid adenomas occurred together. In these three instances of MEA, autopsy did not reveal multiple adenomas in the pituitary and the parathyroids, but this possibly occurred in the pancreas (adenomatosis of the pancreatic islet cells). In another patient with isolated hyperparathyroidism, multiple parathyroid adenomas must be present. Tumor formation in non-endocrine organs was noted twice: a meningioma in one patient (Case III/7) suffering from hyperparathyroidism, and a mediastinal sarcoma in another brother (Case III/4). In addition to the MEA one sister also had a malignant goiter (Langhans).

The frequency of such combinations, and the incidence of adenomas in any one endocrine gland, are given in Tables I and II. The data include all published case reports of MEA, and the three cases which are presented in this paper, thus bringing the total number of reported cases to forty-one.

Of the recorded patients with MEA, tumors of the anterior pituitary were examined histologically in eighteen instances. The adenomas were chromophobe in ten cases, eosinophilic in six cases, and basophilic in one case. Parathyroid adenomas were multiple in more than 50 per cent of the cases [*11*]. Multiple islet cell adenomas were even more frequent. In twenty-three patients in whom the diagnosis was proved surgically or at necropsy, there were fifteen patients with multiple islet cell adenomas, three with adenomatosis, and only five with solitary adenoma.

Table III gives a survey of the frequency of

TABLE III
FREQUENCY OF TUMOR FORMATION IN THE REMAINING ENDOCRINE AND IN NON-ENDOCRINE ORGANS IN THE FORTY-ONE REPORTED CASES (INCLUDING OUR CASES)

Tumor	Cases (no.)
Adrenal adenomas or hyperplasias	10
Goiter	7
Malignant goiter (Langhans)	1
Ectopic islet cells in the duodenum	3
Polyposis of the stomach	3
Pinealoma	1
Bronchial adenoma	1
Fibroepithelial polyp of the tongue	1
Papilloma of the kidney	1
Adenomyoma of the prostate	1
Lipoma (multiple, retroperitoneal)	1

tumor formation in the thyroid, adrenals, and in non-endocrine organs in the cases of MEA that came to our attention. Adenomas of the adrenals and of euthyroid goiter occur so often in the population at large that they are probably of no particular significance in the patients herein described.

As previously noted by Underdahl and Wermer [*9,10*], in most of our cases the hyperparathyroidism was of a mild, non-progressive form. Follow-up studies after several years did not reveal significant lesions in the bones or kidneys. Exploration of the parathyroids was delayed in several patients for this reason.

To the present time, about seventy-five cases of UIA have been recorded. The non-β-cell adenomas were multiple in about 40 per cent of the cases. In six instances, insulin-producing β-cell adenomas were also found. One third of the ulcers are atypically located in the jejunum, postbulbar duodenum or, rarely, in the esophagus. It has also been noted previously that peptic ulcers seem to be more frequent than usual among relatives of patients with UIA [*16*].

The increased frequency of peptic ulcers in patients with MEA has also repeatedly been observed [*6,9–11,13–16*]. The ulcers are usually located in the duodenum; in only two instances were gastric ulcers described. In the thirty-eight cases of MEA reported to date, there was evidence of a concomitant UIA in seven instances.

Several explanations have been considered for the frequent occurrence of peptic ulcers in patients with MEA. In hyperparathyroidism, gastrointestinal ulcers have been noted in 14 to 24 per cent of the cases [*23–26*]. They are often located in the duodenum and usually clear after the hyperparathyroidism is cured. The pathogenesis of these ulcers is not well understood despite experimental studies [*27,28*]. It must be emphasized that in patients with MEA the incidence of peptic ulcers is considerably higher (approximately 35 per cent) than in patients with hyperparathyroidism occurring independently of MEA. Almost 20 per cent of peptic ulcers found in patients with MEA are reported to be of the type of the Zollinger-Ellison syndrome, and 16 per cent are of the commonly encountered type. (Table IV).

The production of excessive amounts of cortisol by adrenal adenomas, as suggested by Fisher [*15*], seems to be of little importance. Functioning adrenal adenomas are rare in patients with MEA, and steroid ulcers, produced by the administration of excessive amounts of glucocorticoids, occur no more frequently in the duodenal than in the gastric portion of the stomach. In addition, peptic ulcers are rarely found in patients suffering from Cushing's syndrome.

TABLE IV
FREQUENCY OF PEPTIC ULCERS IN THIRTY-EIGHT CASES OF MULTIPLE ENDOCRINE ADENOMAS REPORTED IN THE LITERATURE

Type of Gastrointestinal Ulcer	Cases	
	No.	%
Ordinary type of gastric or duodenal ulcer	6	16
Ulcerogenic islet cell adenoma	7	18.5
Total	13	34.5

Hypoglycemia is known to be a stimulus which enhances the secretion of hydrochloric acid and pepsin [*29,30*]. However, peptic ulcers are extremely rare in patients who have been treated with insulin shock, and in patients suffering from hyperinsulinism. Among several hundred cases of hyperinsulinism reported in the literature, peptic ulcers were found in only three [*31–32*].

Excessive production of glucagon from islet cell adenomas has been thought to account for the frequency of peptic ulcers. However, the action of glucagon upon gastric secretion seems to be inhibitory. Although there was stimulation of the gastric mucosa in some studies [*33*], and experimental studies were contradictory [*39*], inhibition was noted in most of them [*34–38*]. Glucagon is certainly not the ulcerogenic factor of the Zollinger-Ellison syndrome [*16*].

In the seven cases reported in the literature in which the classic symptomatology of both MEA and UIA coexisted, it was assumed that a hypothetic ulcerogenic factor was the cause of the ulcers.

The frequent finding of UIA in the reported cases of MEA and the presence of other endocrine disturbances in patients with UIA (Table V), as well as the separate occurrence of both syndromes in siblings (as demonstrated in the present study), favor the concept of a common genetic background for the two conditions. Wermer [*10*] introduced the idea of a dominant inheritance of MEA. This concept is confirmed

TABLE V
FREQUENCY OF ADENOMA FORMATION IN ENDOCRINE ORGANS AMONG SEVENTY-FIVE CASES OF ULCEROGENIC ISLET CELL ADENOMA [*16*]

Type of Adenoma	Cases (no.)
Cortical	10
Pituitary	7
Pancreatic	6
Parathyroid	5
Total	15

by the present study, since consanguinity could be excluded, and the disease was found in subsequent generations. Both MEA and UIA are probably different manifestations of the same gene, which seems to act variably upon the different endocrine organs; these seem to be affected according to the principle of pleiotropy. There is probably a normal penetrance, since symptoms of the disease can be found among all the siblings or their descendents. However, partial expressivity has to be assumed in some instances because of the cases of isolated hyperparathyroidism. Among the thirty-eight cases of MEA, approximately 20 per cent of the patients had symptoms and findings of UIA (which probably represents complete expressivity of the gene).

In the present study it is shown that these two diseases may also occur separately among siblings. It is assumed that partial expressivity of the disease may take the form of isolated hyperparathyroidism, not infrequently found in families with MEA. This form of hyperparathyroidism seems to be different from usual hyperparathyroidism by its mild, non-progressive, chronic course and the frequent presence of multiple parathyroid adenomas.

SUMMARY

A family of seven siblings, of which three sisters suffer from multiple endocrine adenomas, is reported. One had functioning adenomas of the parathyroids and of the β-cells of the pancreas. The second sister suffered from a chromophobe pituitary adenoma and later had hyperinsulinism, while the third had Cushing's syndrome and hyperparathyroidism. Hyperparathyroidism alone was found in one brother and was probably present in three descendents of these siblings. It was found to be a mild form of hyperparathyroidism, with few clinical symptoms, usually producing no lesions in the bones and the kidneys even after many years.

Two brothers among these seven siblings died of atypically located, recurring and perforating peptic ulcers. In one, ulcerogenic islet cell adenoma must be assumed, and it was present, possibly, in the other. Four members of the paternal family died of peptic ulcers, and many others were found to have ulcer complaints.

Among the reported cases of multiple endocrine adenomas, concomitant ulcerogenic islet cell adenoma was often noted, and vice versa.

This study confirms Wermer's concept of dominant inheritance in multiple endocrine adenomas. It must be assumed that the disease entities of multiple endocrine adenomas, ulcerogenic islet cell adenoma, and a particular, familial form of hyperparathyroidism are caused by a dominant, pleiotropic gene which has normal penetrance but variable expressivity.

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Myxedema Following Radioactive Iodine Therapy of Hyperthyroidism*

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RADIOACTIVE iodine has been used in the therapy of hyperthyroidism since 1941 [*1a,1b*], and since 1946 it has been generally available as pile-produced material (I^{131}). During this period one of the most general criticisms of radioiodine therapy of hyperthyroidism has been the incidence of inadvertent induction of myxedema [*2*].

In this study we shall examine the incidence of myxedema as a complication of radioactive iodine therapy for thyrotoxicosis in a large series of patients. The influence of pretreatment, treatment and post-treatment factors will be analyzed in the hope of minimizing this complication in the future.

MATERIAL AND METHODS

Definitions. During the past twelve years the members of the Thyroid Group at The Mount Sinai Hospital have treated over 3,000 hyperthyroid patients with radioactive iodine (I^{131}) in both clinic and private practice. The records of 1,603 of the earliest patients with the longest follow-up period were available for review and analysis. The diagnosis of hyperthyroidism was established by both clinical and laboratory means [*3,4*]. Twenty-four hour I^{131} uptake and/or I^{131} excretion studies were performed in all patients. Blood turnover studies (PBI^{131}) at seventy-two hours and determination of protein-bound iodine (by a modification of the Barker method) were also obtained when indicated [*5*]. Thirteen hundred and ninety-five of these patients were followed-up for at least one year by members of the Thyroid Group. In some instances long-term follow-up data were obtained from other physicians. Appropriate tests, including I^{131} excretion and/or uptake measurements, protein-bound iodine determinations and the basal metabolic rate, were performed when indicated to confirm the final clinical evaluation.

The incidence of myxedema will vary from series to series, depending in part upon the criteria employed in the diagnosis of this condition. In our series the diagnosis was based chiefly on the clinical manifestations, and the necessary laboratory data were obtained only for confirmatory evidence. If determinations of protein-bound iodine and other tests had been performed routinely, it is possible that the incidence of myxedema in our cases might have been higher.

Permanent myxedema is defined for our present purpose as a thyroid deficiency state requiring the use of substitution therapy indefinitely for the alleviation of symptoms. Transient myxedema is defined as a thyroid deficiency state requiring the use of substitution therapy for the alleviation of symptoms, usually for less than six months, followed by restoration of normal thyroid function.

The clinical signs and symptoms of iatrogenic (I^{131}-induced) myxedema do not differ from those familiar in spontaneous myxedema [*6,7*]. There is, however, one striking exception. In the induced disease, almost all patients complain of crampy muscular pain. This complaint is usually spontaneous and often occurs before the characteristic facial changes, skin changes and bradycardia. The pains are most prominent in the muscles of the shoulder girdle and in the legs. They are aggravated by movements that stretch these particular muscle groups, by reaching up for objects or by standing. This complaint is so frequent that it has been used by us as a clinical diagnostic criterion of iatrogenic myxedema. We have no explanation for the high incidence of this complaint in iatrogenic myxedema (at least when induced by radioactive iodine) when contrasted with its lower incidence in spontaneous myxedema. Possibly the quick transition from hyper- to hypothyroid state may result in the prominence of this symptom complex. Others have also commented on the occurrence of this phenomenon after radioactive iodine therapy [*8,9*].

In evaluation of the incidence of myxedema produced by specific forms of therapy of hyperthyroidism it would be helpful to know the exact incidence of this complication in untreated patients with this disease. There are several documented cases in the reported series of untreated patients with hyperthyroidism, but

* From the Department of Medicine, the André Meyer Department of Physics, and the Thyroid Clinic of The Mount Sinai Hospital, New York, New York. Manuscript received October 19, 1960.

TABLE I
THE RELATION OF SEX TO RESULTS OF I^{131} THERAPY OF HYPERTHYROIDISM

Sex	Patients		
	Euthyroid (no.)	Permanent Myxedema	
		No.	%*
Male	286	22	7.1
Female	994	90	8.3

* % here and in all other tables refers to the total number of patients in question.

TABLE II
RELATION OF TYPE OF ONSET TO RESULTS OF I^{131} THERAPY OF HYPERTHYROIDISM

Type of Onset	Patients		
	Euthyroid (no.)	Permanent Myxedema	
		No.	%
Gradual	689	46	6.3
Acute	274	28	9.3
"Hyperacute"	11	0	0
Unknown	302	38	11.2

they are difficult to evaluate. Some of these patients may have had acute or subacute thyroiditis, which may result in the clinical picture of hyperthyroidism, followed by myxedema. Some of the patients in these early "untreated series" received iodine [*10,11*]. It may be concluded that the incidence of myxedema in the course of untreated hyperthyroidism is rare, i.e., less than 1 per cent. It has been disregarded in the discussion that follows.

RESULTS

Of the 1,603 patients in this series 1,252 were euthyroid at the time of their final evaluation; 112 had permanent myxedema; twenty-eight had died of other causes in a euthyroid state; and three were still under treatment for persistent hyperthyroidism. Of the 208 others, twenty-two were lost to follow-up before treatment was complete; 145 were lost to follow-up before final evaluation at one year could be completed; and forty-one died of other causes such as carcinoma, myocardial infarction or accidents. There is no reason to believe that the therapeutic results in these 208 patients would have had a markedly different distribution rate from the main series. Our over-all myxedema rate is 7 per cent; for those followed more than one year it is 8 per cent. In the literature, the reported myxedema rate in patients with hyperthyroidism treated with I^{131} ranges from 3 to 26 per cent [*12,20*].

Pretreatment Factors. We first undertook an analysis of the following pretreatment factors: the effects of age, sex and type of onset upon the final myxedema rate. Other pretreatment factors, such as gland size, duration and type of goiter, were considered and are discussed later.

Sex. In this, as in other large series, the ratio of female patients to male was approximately 4:1. The incidence of myxedema was not statistically different. In other large series, likewise, no significant influence of the sex of the patient in the determination of the incidence of myxedema has been noted [*16–20*]. (Table I.)

Mode of Onset. Prior to this analysis it was our impression that patients with an acute onset of thyrotoxicosis had a higher myxedema rate following I^{131} therapy than patients with a gradual onset. The statistical data bear out this impression. Chapman and Maloof [*16*] made the observation that such patients with acute onset required a lower therapeutic dose; indeed they noted that this type of onset was a determinant of the incidence of myxedema. Two analyses were made to determine if this hypothesis was correct in our patients. First, we analyzed the incidence of permanent myxedema in relation to the type of onset. (Table II.) It will be noted that there was a slightly higher myxedema rate in patients with acute onset than in those with a gradual onset of hyperthyroidism. Since the data are incomplete in about one-third of the cases, they are difficult to interpret with confidence. In addition, we analyzed the effect of duration of goiter and duration of the disease on the myxedema rate. (Tables IIIA and IIIB.) There was a higher incidence of myxedema in the patients with a shorter history of goiter. Shenline and Miller [*17*] found no effect of the duration of disease on the myxedema rate.

Age. It should be noted that in this series all patients above the age of termination of growth, i.e., twenty-two years, were treated with radioactive iodine. There was no preselection of patients by us on the basis of age in adults. Patients under twenty-two years of age were treated only

TABLE IIIA
RELATION OF DURATION OF GOITER TO THE MYXEDEMA RATE IN I^{131}-TREATED PATIENTS WITH HYPERTHYROIDISM

No. of Years	Patients			
	Euthyroid (no.)	Permanent Myxedema		
		No.	%	
<1	291	33	10.2	
1–2	161	4	2.4	
2–3	143	10	6.5	
4–5	57	2	3.4	
6–10	90	5	5.3	
11–15	37	2	5.1	4.9
16–20	34	2	5.6	
21–25	14	2	12.5	
26–30	6	0	0	
>30	22	2	8.3	

TABLE IIIB
RELATION OF DURATION OF DISEASE TO THE FINAL RESULTS OF I^{131} THERAPY OF HYPERTHYROIDISM

No. of Years	Patients			
	Euthyroid (no.)	Permanent Myxedema		
		No.	%	
Unknown	363	45	11.0	
<1	374	34	8.3	
1–2	201	10	5.7	
2–3	170	13	7.1	
4–5	67	3		
6–10	74	2		
11–15	15	3		
16–20	9	1		5.5
21–25	4	0		
26–30	1	0		
>30	2	1		

if there was a definite contraindication to surgery, such as diabetes mellitus or severe cardiac disease, together with a contraindication (agranulocytosis) to antithyroid drugs or failure of antithyroid drug therapy. Twenty-six patients less than twenty years of age were treated with radioactive iodine.

Table IV presents the analysis of age incidence, indicating that younger patients have a higher myxedema rate.

Some evidence has been presented to show that smaller doses may be required in the therapy of hyperthyroidism in the young [*21*]. It was our practice deliberately to undertreat younger patients by administering 20 per cent less than the calculated dose as an initial therapeutic dose. Conversely, it was our practice deliberately to overtreat the aged (over sixty) and thyrocardiac patients, administering 20 per cent more than the initial calculated dose. Despite this, the incidence of myxedema in the younger age groups exceeded that in the older. There may be a greater radiation sensitivity in the young, resulting in a higher incidence of hypothyroidism. Others have noted this influence of age [*22,23*].

TABLE IV
RELATION OF AGE OF PATIENTS AT TIME OF TREATMENT TO RESULTS OF I^{131} THERAPY OF HYPERTHYROIDISM

Age (yr.)	Patients				
	Euthyroid (no.)	Euthyroid Dead (no.)	Permanent Myxedema		
			No.	%	
0–9	3	0	0	0	
10–19	23	0	1	4.2	8.8
20–29	144	0	17	10.6	
30–39	241	2	21	8.0	
40–49	324	2	31	8.7	7.9
50–59	272	6	21	7.0	
60–69	178	10	11	5.5	5.3
70–79	37	6	2	4.4	
80–89	3	0	1	...	

Eye Signs. We analyzed our data to determine whether the presence of exophthalmos and/or lid signs had any specific effect upon the myxedema rate. There is no clear indication of any change or influence on the myxedema rate in patients with exophthalmos as contrasted with the control group. Beierwaltes and his group [*24*] also found the same myxedema rate in patients with exophthalmos as in their control group.

Our experience does not substantiate the widespread clinical opinion that the development of myxedema by ablation of the thyroid either by surgery or by I^{131} therapy may be deleterious to the eyes. Of twenty-eight patients with exophthalmos prior to treatment in whom

permanent myxedema developed, only one showed some change for the worse in the condition of his eyes.

Dosage Analysis. The action of the isotope depends, of course, upon the destruction of functioning thyroid tissue by internal radiation. The dosage of radioactive iodine is so chosen that a relatively high proportion of patients will have a remission after one or two treatments, without too high an incidence of hypothyroidism. Four basic schools of thought have developed concerning selection of the individual treatment dose to accomplish this goal. All four have their virtues and faults [*22,23*].

(1) A fixed dose is administered to all subjects. (2) The dose is adapted to the weight of the gland, i.e., a set number of microcuries per estimated gram of thyroid weight. (3) The dose is adapted to the weight of the thyroid and the uptake of the isotope in the gland; a dose of I^{131} expressed as microcuries retained per gram of estimated thyroid weight. (4) A predetermined amount of radiation is given; an amount of I^{131} sufficient on the basis of thyroid weight and the elimination and decay of the isotope in the thyroid to deliver a predetermined dose of radiation to the gland (expressed in rads).

The most general criticism has been directed toward the first, or standard dose method. This criticism is summarized by Larsson [*23*] as follows: "The first method can hardly be used in a series with goiters varying greatly in size, since it is impossible to choose an activity suitable for small goiters which may produce remission in large goiters within a reasonable period of time, even after multiple treatments."

Most investigators have used dosage schedules in accordance with the second or third basic schemes outlined. In principle, these schools differ but little, since a majority of toxic goiters have a high uptake. In practice it is even more difficult to distinguish between these two schools, since most groups increase the administered dose to compensate for the rare hyperthyroid patient with a low uptake when using the principle advocated under the second scheme. The greatest disadvantage here is that these variable dose methods are dependent on accurate estimation of the weight of the thyroid gland. Palpation, while crude, is the most commonly used method. Certain variations and errors are inherent in this use of palpation as a method of estimating gland weight. The weight of a bulky but soft vascular gland is likely to be underestimated. In young women with considerable anterior curve of the cervical vertebrae, the gland, although small, may be easily seen and felt, and this may lead to overestimation of the weight. Conversely, in male patients with heavy, thick, musculature of the neck, underestimation of the weight of the gland may occur and a failure of therapy may ensue. Occasionally the delineation of some projection of the thyroid gland [*25*] with x-ray and pneumoradiography [*26*] or by scanning after I^{131} [*4*] administration may be helpful in the estimation of size [*27*]. Obviously, estimates of thyroid weight vary from observer to observer.

On a theoretic basis, the fourth method appears to be the most rational. It takes into account not only the variation in size of the gland but also the variations in effective half-life of the administered I^{131}. However, the changes induced by treatment may cause variations in delivered dosage significantly different from the doses calculated [*28*].

It should be noted that none of these schemes for dose estimation can altogether take into account the variability from patient to patient because of possible differences in cellular sensitivity to radiation or differences of distribution of the isotope within the thyroid gland. This latter factor may result in higher than calculated radiation doses.

In the present series the third method was employed as a basic dosage scheme. Originally we attempted to deliver an initial dose of 100 μc. of I^{131} retained per estimated gram of thyroid weight.* Initially, thyroid weights were correlated with the actual weight of operative and autopsy specimens. As noted by Williams [*15*], there may be startling variations, but in general the palpatory results in our hands were as "good" as those reported by him. The calculated basic dosage scheme outlined was altered for various reasons: (1) Age: In persons under the age of forty, the initial dose administered was 20 per cent less than the calculated dose, since we considered that the deleterious effects of permanent myxedema were more to be feared than possible slight prolongation of the hyperthyroid state. In persons over the age of sixty, the initial

* The "millicurie" used originally was based on a local standardization. When a reproducible unit was established by the National Bureau of Standards it turned out that our "millicurie" contained 10 per cent more disintegrations per second than the standard. The statements on dosage have been corrected for this error, and the millicuries indicated agree presumably with the present standardization of the NBS.

TABLE V
RELATION OF TYPE OF GLAND TO RESULTS OF I^{131} THERAPY OF HYPERTHYROIDISM

Type of Gland	Patients		
	Euthyroid (no.)	Permanent Myxedema	
		No.	%
Diffuse	872	70	7.4
Single nodule	97	12	11.0
Multi-nodule	216	18	7.7
Substernal	38	0	0
Total	1,223	100	7.6

dose was usually 20 per cent higher than the calculated dose, since it was our opinion that myxedema was less harmful than continued hyperthyroidism in this age group. (2) Previous surgery: In patients with previous surgery, unless the thyroid gland was clearly palpable and the palpable gland determined to be functioning by means of suitable scanning procedure, no more than 2 mc. of I^{131} were administered as an initial therapeutic dose. (3) In patients with nodular goiters, the initial dose was calculated for a retained dose of 120 μc. per estimated gram of thyroid weight. (4) In the presence of diabetes mellitus or severe cardiac disease, the initial dose was approximately 20 per cent more than the calculated dose. Deliberate overtreatment was given in severe thyrocardiac subjects, in which case the calculated initial dose was usually twice that of the normal subjects.

Depending upon the patient's clinical response to therapy, second or subsequent doses of radioactive iodine were administered eight to sixteen weeks after the initial treatment. A long interval of time, as much as four months following the initial I^{131} therapy, may be required before the full improvement from this initial therapeutic dose is apparent. If retreatment is performed "on an improving curve," there will be a higher incidence of myxedema [*17*]. Therefore, if the patients showed continued clinical improvement no additional therapy was given until a total post-therapeutic period of sixteen weeks had elapsed. If, however, the patient was still severely thyrotoxic and the I^{131} uptake study at twenty-four hours was still elevated, a second therapeutic dose was given after as short a period as eight weeks. Subsequent therapeutic doses were delivered at similar intervals.

The size of these subsequent therapeutic doses varied, depending on the clinical response. Usually, the second dose was calculated in the same manner as the initial dose. If, however, marked clinical improvement had occurred, only half of the calculated dose was administered. On subsequent doses, similar calculations were used. Occasionally, in the face of persistent severe hyperthyroidism with no response to two or more therapeutic doses, much more than the calculated dose (two or three times) was given as a third or fourth dose.

Effect of Type of Gland. It is the policy of some institutions not to treat patients with toxic nodular goiters with radioactive iodine [*17*]. Some of those who favor the surgical approach do so because they believe that the response of the patient with toxic nodular goiter to radioactive iodine therapy is poor, and that the risk of carcinoma is greater. Crile, McCullagh and Glasser [*29*] suggested that the incidence of myxedema in patients with diffuse goiters would be significantly higher than in those with multinodular goiters. In this series, however, the prevalence of myxedema was equal in patients with diffuse and multinodular goiters. Unlike most investigators, we find that the presence of palpable "nodules" did not generally affect the response to therapy. The highest incidence of myxedema was found in patients with a solitary nodule. (Table v.) Some of these may represent unilateral hyperplasia in a diffuse toxic goiter.

Analysis of this group (the solitary nodular goiter) showed that all the patients had initial estimated thyroid weights of 40 gm. or less. Perhaps in those patients in whom permanent myxedema developed, the solitary nodules contained most of the functioning thyroid tissue. (Almost all of these nodules on profile were "hot," i.e., functioned more than the remainder of the gland.)* In the estimation of the weight of the gland it was assumed that, in addition to the solitary nodule, the remainder of the thyroid gland was "normal" in weight. This assumption may have been in error and may have led to

* A full discussion of the treatment of toxic nodular goiters in this series is contained in: Eller, M., Silver, S., Yohalem, S. B. and Segal, R. L. The treatment of toxic nodular goiter with radioactive iodine. *Ann. Int. Med.*, 52: 976, 1960.

TABLE VI
RELATION OF THE INITIAL ESTIMATED WEIGHT OF THE THYROID GLAND TO RESULTS OF I^{131} THERAPY OF HYPERTHYROIDISM

Grams	Patients			
	Euthyroid (no.)	Euthyroid Dead (no.)	Permanent Myxedema	
			No.	%
<30	223	5	33	12.6
31–40	319	6	31	8.7
41–50	307	7	21	6.3
51–60	147	2	6	3.9
61–70	40	0	0	4.4
71–80	67	0	6	
81–90	19	1	1	
91–100	38	0	0	
101–110	1	0	0	
>111	28	2	2	

TABLE VII
RELATION OF TOTAL NUMBER OF MILLICURIES ADMINISTERED TO THE FINAL RESULTS OF I^{131} THERAPY OF HYPERTHYROIDISM

Total No. of Millicuries	Patients			
	Euthyroid (no.)	Euthyroid Dead (no.)	Permanent Myxedema	
			No.	%
1	0	0	0	0
2	29	0	7	19.4
3	97	1	8	7.5
4	211	1	30	12.4
5	187	5	24	11.1
6	143	5	15	9.2
7	97	1	4	4.4
8	111	2	9	
9	72	2	6	
10	49	5	2	
11	38	0	0	
12	35	0	1	
13	23	0	1	
14	16	2	1	
15	19	0	0	
16	17	0	1	
17	12	1	0	
18	11	1	1	
19	11	0	1	
20	1	0	0	
21	8	0	0	
22	4	0	0	
23	4	0	0	
24	4	0	0	
25	4	0	0	
26	5	0	0	
27	5	0	0	
28	3	0	0	
29	26	2	1	
30	10	0	0	
unknown	1			

overestimation of the weight of the gland, with consequent overdosage. That the error occurred only in smaller glands would be consistent with this explanation.

Effect of Initial Gland Size. Our incidence of myxedema was greatest when the estimated gland was in the lower size range. (Table VI.) Since the administered dose was based on the estimated weight of the gland, we found the highest incidence of myxedema inversely proportional to the total number of millicuries administered. (Table VII.) It would seem that we tended to overestimate the weight of the smaller glands. A relatively small error in overestimation of the size of the small gland would result in a larger increment in the administered dose of I^{131} than the same error in a larger gland.

Effect of Number of Administered Therapeutic Doses. We believe that the greater the inherent radiosensitivity of the thyroid, as shown by the fewer number of therapeutic doses needed, the higher the myxedema rate. The incidence of myxedema was 12 per cent for patients given only one therapeutic dose; 6 per cent for those given two doses; 4 per cent for those given three doses; and there were no instances of permanent myxedema in patients requiring more than five therapeutic doses. (Table VIII.)

Effect of Previous Treatment of Hyperthyroidism. Since previous therapeutic procedures may influence the size of the thyroid gland and therefore the dose of I^{131} administered and the dose of radiation delivered to the gland, and furthermore may alter the sensitivity of the thyroid to radiation, the effects of previous therapy of hyperthyroidism must be taken into account.

The administration of antithyroid drugs is known to cause hyperplasia of the gland with a consequent increase in the number of cells, an increase in mitosis, and an absolute increase in gland size. Conflicting results have been noted on the effect of prior antithyroid drug therapy upon I^{131} treatment results and dosage [*15–17*]. Previous iodine therapy usually has no effect on the amount of radioactive iodine required [*15–17*].

TABLE VIII
RELATION OF TOTAL NUMBER OF TREATMENTS TO THE FINAL RESULTS OF I^{131} THERAPY OF HYPERTHYROIDISM

Total No. of Treatments	Patients			
	Euthyroid (no.)	Euthyroid Dead (no.)	Permanent Myxedema	
			No.	%
1	575	15	81	12.1
2	357	8	22	5.7
3	169	5	8	4.4
4	86	0	1	1.1
5	25			
6	17			
7	9			
8	6	0		
9	3			
10	0			
11	2			
12	1			

In patients with previous subtotal thyroidectomy for hyperthyroidism, the remaining thyroid tissue undergoes hyperplasia and hypertrophy. In many of these patients accurate estimation by palpation of the weight of the thyroid gland may be difficult if not impossible. The scar tissue itself will give a false overevaluation of the estimated thyroid weight. This overestimation of the thyroid weight by palpation will result in overtreatment with I^{131} when the dosage is based on presumptive thyroid weight.

Analysis of our patients in regard to previous therapy gave the following results: 552 patients had no known prior therapy; in fifty-two (9 per cent) permanent myxedema developed. In those patients who had been previously treated with antithyroid drugs, iodine, or a combination of these forms of therapy, the incidence of myxedema was lowest, i.e., 3 to 4 per cent. We can only surmise that previous therapy with either iodine or antithyroid drugs, or with a combination of these drugs, may decrease the radiosensitivity of the gland.

In regard to surgery, our initial impression that patients with previous thyroid surgery would have a high myxedema rate was substantiated in the patients who had undergone surgery once or twice prior to I^{131} therapy. This initial impression stemmed from our early experiences in which five of the first seven previously thyroidectomized patients with recurrent hyperthyroidism had permanent myxedema following treatment with radioactive iodine. Our alteration of dosage based on this experience may have been responsible for the lower incidence of myxedema in this total series. The highest rates of myxedema were found in those patients who had had previous therapy involving permanent destruction of a large proportion of the thyroid gland, i.e., x-ray and/or multiple operations.

These findings are noted in Table IX. The one startling statistic is the high incidence of myxedema in previously untreated patients with thyrotoxicosis as compared with those who had had previous non-destructive medical therapy for hyperthyroidism.

Other Factors of Note. The presence of diabetes mellitus had no effect on the myxedema rate. In only two of the sixty-six patients with diabetes mellitus did permanent myxedema develop.

The over-all incidence of myxedema in our cardiac patients was the same as in the rest of the series. However, in view of the larger doses deliberately administered to patients with very severe heart failure or angina pectoris, the

TABLE IX
RELATION OF PREVIOUS THERAPY OF HYPERTHYROIDISM TO THE RESULTS OF I^{131} THERAPY OF HYPERTHYROIDISM

Type of Therapy	Patients		
	Euthyroid (no.)	Permanent Myxedema	
		No.	%
None	500	52	9.4
Surgery	71	5	6.6
Antithyroid drugs	315	14	4.3
Iodine	111	5	4.3
Antithyroid drugs and iodine	105	3	2.8
Antithyroid drugs and destructive therapy, i.e., x-ray and/or surgery	42	8	16.0
Iodine and destructive therapy, i.e., x-ray and/or surgery	13	5	27.8
Antithyroid drugs, iodine, and destructive therapy, i.e., x-ray and/or surgery	17	4	19.0
Unknown	102	15	12.8

incidence of myxedema was significantly higher in this group, i.e., 14 per cent.

Post-Therapy. In this series the patients were not routinely given any medication after I^{131} treatment. Approximately 40 per cent of our patients were given iodide, usually starting seventy-two hours after therapy and continuing for three to four weeks. This therapy was given to those patients who, in our opinion, could not tolerate the deleterious effects of long-continued hyperthyroidism, since there is a lag period of three to four weeks after the administration of I^{131} before any effect is noted. Some groups [*20,30*] have administered antithyroid drugs after I^{131} therapy to alleviate the symptoms of hyperthyroidism.

Duration of Follow-Up. Analysis of the series by year of initial treatment (Table x) shows a lower myxedema rate for the later years. There is one year, 1951, in which this trend is not present. During that year we made the mistake of retreating some patients solely on the basis of uptake and high blood I^{131} turnover measurements. We know now that previous therapy may give PBI^{131} plasma levels in the hyperthyroid range in cured patients [*4*].

The patients treated earlier, of course, had the longer follow-up periods, consequently the highest incidence of myxedema was noted in the patients with longer follow-up. (Table xi.) It is possible, to be sure, that the total incidence of permanent myxedema actually increased with the passage of time. Others have reported this, but examination of our data showed that the onset of clinical myxedema usually was noted during the first eight months following the last therapeutic dose. We have seen a few patients in whom myxedema occurred as late as thirty-six to forty-two months after I^{131} treatment. However, the number of these "late myxedema patients" is relatively small; in only four patients did myxedema develop more than twelve months after the last I^{131} treatment. On the other hand, some euthyroid patients, because of their symptom-free state, did not return for long-term follow-up evaluation, whereas the patients with myxedema were more likely to attend follow-up clinics because of the presence of myxedema and their resultant symptoms. This unavoidable selection in follow-up would weight the statistics in favor of a higher incidence of myxedema with passage of time.

We are inclined to the more optimistic interpretation and to the belief that the steadily diminishing myxedema rate represents improvement in our technic. Larsson [*22,23*], Shenline and Miller [*17*], and Clark and Rule [*13*] also noted a lower myxedema rate in their more recently treated patients. They also ascribe this improvement to improvement in their technic.

TABLE X
RELATION OF THE YEAR OF INITIAL I^{131} THERAPY TO RESULTS OF I^{131} THERAPY OF HYPERTHYROIDISM

Year	Patients			
	Euthyroid (no.)	Permanent Myxedema		
		No.	%	
1947	7	2	22.2	16.3
1948	37	7	15.9	
1949	95	18	15.9	
1950	185	15	7.5	
1951	271	29	9.7	
1952	278	21	7.0	
1953	231	13	5.3	
1954	175	7	3.8	

TABLE XI
RELATION OF LENGTH OF FOLLOW-UP IN YEARS TO RESULTS OF I^{131} THERAPY OF HYPERTHYROIDISM

Years	Patients		
	Euthyroid (no.)	Permanent Myxedema	
		No.	%
1.0	229	11	4.6
1.5	142	13	8.4
2.0	188	10	5.1
2.5	146	9	5.8
3.0	157	15	8.7
3.5	84	8	8.7
4.0	118	12	9.2
4.5	54	5	8.5
5.0	59	5	7.8
More than 5	110	24*	17.9

* All but ten were treated before 1951.

TRANSIENT MYXEDEMA

There were 103 patients (7 per cent) in whom transient myxedema developed. Tran-

TABLE XII

FREQUENCY OF PERMANENT MYXEDEMA FOLLOWING I^{131} THERAPY OF HYPERTHYROIDISM

Author	Patients			Dose	Comments
	Hyperthyroid (no.)	Permanent Myxedema			
		No.	%		
Group I. Patients Treated with a Fixed Dose (See Text)					
Gordon, Albright [31]	120	3	2.4	3.0 mc.	Some variation with gland size
Balls et al. [9]	180	33	18.3	6–10 mc.	. . .
Group II. Patients Treated with a Fixed Administered Dose of I^{131} per Gram (See Text)					
Williams et al. [15]	97	3	3.0	150 μc. per gm.	Pretreatment with propylthiouracil
Clark, Rule [13]	628 (262 diffuse, 229 nodular)		17 (incl. transient)	150 μc. per gm. diffuse 300 μc. per gm. nodular	137 patients, recurrence after thyroidectomy
Beierwaltes, Johnson [24]	330 (248 diffuse, 82 nodular)	45	15 (incl. transient)	Approx. 250 μc. per gm.	. . .
McCullagh [19]	642	?	?	100–200 μc. per gm.	10% myxedema, including transient
Shenline, Miller [17]	431	70	17	120 μc. per gm. diffuse 240–360 μc. per gm. nodular	17% myxedema, including transient
Sweeney et al. [33]	111 (83 diffuse, 28 nodular)	34	23.4	200 μc. per gm.	. . .
Werner et al. [20,32]	525	78	14.8	Approx. 100 μc. per gm.	Antithyroid drugs after treatment
Larsson [22,23]	351	44	11.1	Approx. 100 μc. per gm.	. . .
Group III. Patients Treated with a Specific Retained Dose of I^{131} per Gram (See Text)					
Chapman, Maloof [16]	520	41	7.9	100 μc. per gm.	. . .
Ward, Skillern, Cook [34]	183	10	5.2	100 μc. per gm.	. . .
Myant [35]	59	6	9.2	100 μc. per gm.	. . .
Mount Sinai Hospital (present series)	1603	112	7.0	100 μc. per gm.	. . .
Group IV. Patients Treated with a Calculated Amount of Radiation to Gland (See Text)					
Bauer, Blahd [14]	106	6	5.4	7,000 r	Weight calculated from scintogram
Cassidy, Astwood [30]	200	29	14.5	10,000 r	. . .
Blomfield [36]	133	15	10.1	6,000–8,000 r	. . .

sient myxedema was defined as hypothyroidism severe enough to be symptomatic, which then spontaneously disappeared. Of these 103 patients, sixteen (15 per cent) had hypothyroidism of more than six months' duration. Only six of this group had hypothyroidism of more than eighteen months' duration. All these patients, at the time of their final evaluation, were euthyroid without thyroid medication.

COMPARISON WITH OTHER LARGE SERIES OF I^{131}-TREATED PATIENTS WITH HYPERTHYROIDISM

Analysis of the larger reported series of I^{131}-treated patients with hyperthyroidism in respect to the incidence of post-therapy myxedema is presented in Table XII. These reports are grouped according to the "basic dosage philosophy" used (*vide supra*). It is apparent that there is a marked variation in the incidence of myxedema: from a low of 3 per cent to a high of 26 per cent. In general, those reporting higher myxedema rates administered larger amount of I^{131}. Because of a relatively high myxedema rate, some groups have changed from the fixed dose method to the variable dose method and some of those using a higher administered dose of I^{131} have reverted to smaller doses [*34*].

SUMMARY AND CONCLUSIONS

The incidence of myxedema in a series of 1,603 patients with hyperthyroidism treated with radioactive iodine is reported. The doses of I^{131} administered were calculated on the basis of approximately 100 μc. of I^{131} retained per gram of estimated thyroid weight.

One hundred and twelve patients (8 per cent) were found to have myxedema at the time of their final evaluation one to eight years after the completion of therapy; 1,252 were euthyroid. (In 103 additional patients transient myxedema developed which did not require the permanent use of supplemental thyroid medication.) The incidence of myxedema cited in other series reported varies from 3 to 26 per cent. Analysis of the factors influencing the myxedema rate showed that the incidence of myxedema was higher: (1) in young patients, (2) when I^{131} was given after previous surgery or x-ray therapy, (3) in patients with smaller glands, (4) in those with short duration of disease or goiter, (5) when a smaller number of doses of radioiodine was required for cure. The following factors tended to lower the incidence of myxedema: (1) previous therapy with antithyroid drugs or iodine, (2) large thyroid glands, (3) larger number of doses required for cure.

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Bacterial Infection and Some Effects of Chemoprophylaxis in Chronic Pulmonary Emphysema*

I. Chemoprophylaxis with Intermittent Tetracycline

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ACUTE episodes with many of the characteristics of infection occur frequently in the course of chronic pulmonary emphysema and are a major cause of death, increased respiratory insufficiency and congestive heart failure in patients with this disease. Although the exact etiology of many of these episodes cannot be proved, they have generally been attributed to bacterial infection and have been called by names that suggest an infectious or inflammatory origin, e.g., attacks of bronchitis, acute exacerbations of bronchitis, bronchial infections, and bronchopneumonia. The role which these acute episodes play in the pathogenesis of chronic pulmonary emphysema is not clear, but bronchial and bronchiolar abnormalities with pathologic findings suggestive of an inflammatory origin have commonly been observed [*1–5*].

Numerous investigators have carefully studied chronic bronchitis and the superimposed acute episodes in an attempt to determine the importance of infection in that syndrome, but the results have often been conflicting. Furthermore, epidemiologic and environmental studies have suggested that chronic bronchitis and emphysema, as they are observed in Great Britain, may be different from chronic pulmonary emphysema in the United States [*6–8*]. A study of bacterial infection and the effect of a chemoprophylactic regimen was therefore undertaken in a group of patients with emphysema observed in an urban American clinic. A control group was used to evaluate the effect of prolonged chemotherapy on (1) their subjective feeling of well-being, (2) the incidence of clinical evidence of infection of the lower respiratory tract, (3) the gross characteristics and the bacterial flora of the sputum, and (4) changes in pulmonary function.

REVIEW OF PREVIOUS OBSERVATIONS

There have been many studies of the bacterial flora in the sputum of patients with chronic bronchitis or emphysema between and during acute exacerbations. In a few of these, attempts to establish a viral etiology of the exacerbations have been made; in others, pathologic specimens of bronchi have been examined. The effect of therapy with chemotherapeutic drugs in such patients has also received much attention.

Nearly all investigators have found a wide variety of microorganisms in the sputum of patients with chronic bronchitis, but until recently most of them have not been able to identify microorganisms that seemed to be the cause of the chronic infection or the acute exacerbations. Marshall [*9*] reported that pneumococci, streptococci, Micrococcus catarrhalis or Bacillus Friedländer were found in nearly every case. Southwell [*10*] described a similar mixture and mentioned no predominance of any microorganism. In a study of fifty patients with

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chronic bronchitis, Howell [*11*] found M. catarrhalis, Streptococcus viridans, pneumococcus, B. Friedländer and Hemophilus influenzae to be the predominant microorganisms in most cases. Other bacteria very rarely made up the principal flora.

Studies of bronchial aspirates similarly have been of little help in revealing the etiology of chronic bronchitis or the exacerbations. Allison and his associates [*12*], in twenty-six cases of chronic non-purulent bronchitis, found more than half of the bronchial specimens to be sterile while the remainder yielded a variety of microorganisms, not including H. influenzae. Seven of eleven bronchial specimens from patients with tracheitis and bronchitis associated with emphysema studied by Benstead [*13*] were also found to be sterile. Pecora and Yegian [*14*] in a variety of pulmonary diseases, including some cases of chronic bronchitis, studied the bacterial flora of expectorations, bronchoscopic aspirates and cultures of exposed tissue at thoracotomy; they concluded that the lower respiratory tract harbors pyogens relatively rarely. They therefore believed that what is frequently diagnosed as chronic bronchitis is due more to inspired irritants such as cigarette smoke and industrial fumes than to chronic infection with pyogenic bacteria.

More recently, using a new technic of bronchial swabbing, Lees and McNaught [*15*] found predominently H. influenzae and pneumococcus in the bronchial secretions of patients with chronic bronchitis while the bronchial secretions of "normal" subjects were sterile. They suggested, on the basis of their work and that of Brumfitt [*16,17*], that the finding of such microorganisms in sputum was not significant as they could represent mechanical contamination from the oropharynx.

In contrast, as early as 1938 Mulder [*18*], working with natives and Europeans in the Dutch East Indies and in the Netherlands, found that a single predominant microorganism could usually be isolated, that the sputum rarely contained more than three microorganisms, and that H. influenzae was present in 96 per cent of the cases of purulent bronchitis. He concluded that this organism played an important role in chronic bronchitis and was the most probable etiologic agent for the suppuration of the bronchial mucous membrane. In subsequent articles [*19,20*] he and his associates confirmed these earlier observations and also concluded that pneumococcus was next in importance to H. influenzae. Mulder used multiple cultures from each specimen, and employed special media to favor the growth of H. influenzae. H. influenzae recovered by him, and by later workers who have studied the sputum or bronchial specimens from patients with chronic bronchitis, has generally been of the non-encapsulated variety.

Later, May [*21*] at the Brompton Hospital in London demonstrated that microorganisms are inhomogeneously distributed in the sputum of patients with chronic bronchitis, and suggested that this could account for some of the disparate results which had previously been reported. He recommended that either multiple cultures be taken randomly from many different parts of the sputum specimen, or that the material should be homogenized according to a method of pancreatin digestion that was devised by Rawlins [*22*]. May demonstrated that although many different microorganisms were present in the sputum there was a significant association between the presence of pneumococcus and H. influenzae and pus in the sputum. In a subsequent article [*23*] he concluded that because purulency tends to disappear with the elimination of pneumococcus or H. influenzae and recurs with their reappearance, these two organisms seem to be "far more important than any others in chronic bronchitis." A study by Helm et al. [*24*] confirmed these previous findings of May.

Stuart-Harris and his associates [*25*] at Sheffield, in studying the factor of infection in chronic bronchitis, found pneumococcus, H. influenzae, and Staphylococcus aureus to be frequent inhabitants of the sputum in all phases of the disease and they postulated that the bronchi may become autogenously infected by nasopharyngeal microoorganisms. The finding by Brown, Coleman, Stranahan and Stuart-Harris [*26*] in Albany of fewer pathogenic bacteria in bronchoscopic specimens than in sputum was thought to be due to failure of a single aspirate to represent all areas of the lung, and was not interpreted to mean that sputum organisms were derived by mechanical contamination from the nose and throat. In another study, Clifton, Pownall and Stuart-Harris [*27*] found a high incidence of pneumococci but could not relate them etiologically to acute exacerbations.

At about the same time, Elmes, Knox and Fletcher [*28*] at the Hammersmith Hospital in London found a similar relationship between

purulent sputum and the presence of pneumococcus or H. influenzae, and in addition observed that fog tended to increase sputum purulence in patients with H. influenzae. In a later article [*29*] they stated that H. influenzae was important but were uncertain as to its exact role in the acute exacerbations of chronic bronchitis.

In 1957 Edwards and his associates [*30*], reporting from Leeds, also found H. influenzae and pneumococcus to be the most commonly occurring pathogens in their series of 320 cases. Like May, they found H, influenzae to be the major microorganism associated with purulent sputum, but like Knox et al., in respect to acute exacerbations, they could not, on the basis of their study, state whether either pneumococcus or H. influenzae was responsible for maintaining the symptom complex of chronic bronchitis.

Although most investigators have been concerned with the bacterial factors in chronic bronchitis and its exacerbations, a few have suggested that viral infections may contribute to the evolution of this disease. Stuart-Harris and his associates [*25*] detected influenza virus infection mainly in relation to the acute exacerbations of chronic bronchitis, but a rise in influenza antibody titers was not invariably accompanied by clinical relapse. In patients with chronic bronchitis without congestive heart failure they found influenza virus A frequently to be a cause of clinical relapse sufficiently severe to necessitate admission to the hospital, but in the group with congestive heart failure, influenza viruses seemed less often to cause clinical relapse. The period of study included two epidemics of influenza A virus. These investigators postulated that viruses probably disturb the distribution as well as the quantitative relationships of the bacterial flora and that they probably also hinder normal protective mechanisms mechanically by parasitizing host cells and causing increased mucous secretion. They concluded that in chronic bronchitis there is a failure of defense of the lower respiratory tract against invasion by nasopharyngeal organisms and that repeated attacks of respiratory viruses may be instrumental in bringing this about. In a subsequent article [*31*] and monograph [*32*], Stuart-Harris reiterates this view.

Oswald [*33*], in discussing factors in pathogenesis of chronic bronchitis, stated that although it is not known whether viruses are concerned in the original attack of bronchitis, they are commonly associated with relapses.

On the other hand, Murdoch and his coworkers [*34*] who, in the course of evaluating continuous antibiotic therapy in chronic bronchitis, performed antibody titers to influenza A, B and C viruses and to the adenovirus group, observed that relatively few patients had increased antibody titers to viruses. Even when these were present often they were not associated with clinical changes, a finding that was interpreted to mean that patients with chronic bronchitis are able to withstand virus infections without the possible serious consequence of an acute exacerbation clinically.

Hers and Mulder [*35*] examined the histologic structure of respiratory tract epithelium in both autopsy and surgical specimens from patients with either acute or chronic mucopurulent bronchitis believed to be due to H. influenzae, in an attempt to determine whether H. influenzae is a primary cause of infection or a secondary invader to a virus infection. H. influenzae was demonstrated most frequently between the epithelial cells of the bronchi or bronchioles and occasionally below the basement membrane. Leukocytic infiltration between cells was noted but the epithelial cells in both the acute and chronic cases were intact. As it is known that influenza virus produces necrosis down to the basal cell layer of the tracheal and bronchial epithelium, the finding of intact epithelium suggested to these workers that the acute cases, at least, must represent primary hemophilus infection without initiation by a respiratory virus. Hers and Mulder, nevertheless, postulated that viral infections (such as influenza and measles) causing necrosis of the mucus-producing ciliated cells in the epithelium, most probably promote the entry of hemophilus into the mucosa.

Whether or not it is due to bacterial or viral infection, the inflammatory nature of chronic bronchitis has been clearly demonstrated histologically by Reid [*1*]. In addition to hypertrophy of the mucus-secreting elements (the principal abnormality found in the early phase of the disease), she described in advanced cases such changes as infiltration of bronchiolar walls with acute inflammatory cells, abscess formation, plugging of lumens with pus and mucus, and dilation. She cites these acute inflammatory changes as evidence of the presence of infection, and the resultant disorganization of secondary lobules as an indication of the importance of infection in the progression of chronic bronchitis.

Many of these investigators and others have studied the effects of chemotherapy on some aspects of chronic bronchitis. One of the earliest reports of systematic therapy of bronchitis with different antibiotics is by Mulder [*19*] who, in short-term treatment of 125 patients with mucopurulent bronchitis and H. influenzae in their sputum, found that with sufficiently high doses of various antibacterial drugs it was possible in most patients to eliminate H. influenzae from the sputum; coincident with this effect there was a disappearance or decrease in clinical symptoms of bronchitis. He concluded then that H. influenzae must have been the cause of the inflammation in these patients.

May [*23*], in a similar effort to evaluate the pathogenicity of certain microorganisms (particularly H. influenzae and pneumococcus) in chronic bronchitis, studied the short-term effect of four different antibiotics on the purulence, volume and flora of the sputum in twenty-two patients with purulent or mucopurulent sputum and seventeen patients with mucoid sputum. In every patient with purulent sputum, antibiotic therapy was found to eliminate or greatly reduce the pus and usually to decrease the volume of sputum. However, many patients felt subjectively worse when their sputum was rendered mucoid possibly because of increased tenaciousness of the secretions. H. influenzae and pneumococcus were the microorganisms most closely correlated with pus, their eradication with drug therapy being accompanied by disappearance of pus and their reappearance with a return to purulence. Among the patients with mucoid sputum, antibiotic therapy had no appreciable effect on the character or volume of the sputum even when pathogenic bacteria had been eliminated. In a later report, May [*36*] distinguished between neutrophil and eosinophil pus and emphasized a previous finding of Helm and his co-workers [*24*] that antibiotic therapy was of most clinical value when polymorphonuclear neutrophils predominated in the sputum.

Stuart-Harris and his co-workers [*25*], Elmes et al. [*28*], and Knox and her associates [*29*] observed independently that antibiotic therapy exerts a definite effect on the quality and quantity of sputum in relation to their effects on the bacterial flora, particularly H. influenzae and pneumococcus, but from their small series Knox et al. concluded that penicillin and streptomycin aerosol therapy could not be relied upon to control bronchial infection by H. influenzae. Most investigators have emphasized the difficulty of eradicating H. influenzae and the frequency of bacterial and clinical relapse upon cessation of drug therapy.

One of the earliest studies of the effects of long-term chemoprophylaxis in chronic bronchitis to employ control subjects who were taken at random and simultaneously treated with placebos was carried out by McVay and Sprunt [*37*] at the University of Tennessee. Using chlortetracycline and identical placebos for a period of two weeks to twenty months, they found that among the drug-treated group the severity of acute infections of the respiratory tract was reduced and the incidence cut by more than 50 per cent, that purulent sputum was rare and that the patients felt subjectively better. Regular bacteriologic examinations were not performed.

In 1956 Helm, May and Livingstone [*38*] reported on the effects of long-term treatment with oxytetracycline on various chronic respiratory infections in a group of patients, including seventeen with chronic bronchitis. They stated that antibiotic therapy is beneficial for long periods (up to at least two years) and that the beneficial effects are probably due to suppression of pneumococcus and H. influenzae. H. influenzae was not completely eradicated, but no pneumococci were isolated during therapy. In the same year, May and Oswald [*39*] published their results with six months of oxytetracycline or tetracycline therapy in thirty-seven patients with advanced chronic bronchitis. Although they stated that before therapy H. influenzae was persistently present in every patient and that after therapy about a quarter of the patients harbored resistant Staph. aureus, they did not otherwise give detailed bacteriologic results. They noted that 59 per cent of the patients showed improvement as evidenced by a decreased number of acute infections of the respiratory tract (as determined by increased pus, fever and days off from work), and a diminution in sputum purulence and volume. No simultaneous control studies were carried out.

In 1957 Elmes, Fletcher and Dutton [*40*], using a simultaneously-treated control group attempted to determine if a short course of antibiotic therapy at the onset of an exacerbation could reduce the duration of the episode. They studied eighty-eight outpatients with chronic bronchitis for a period of months and found that although the incidence of H. influenzae was

reduced by two-thirds and no pneumococci were isolated during treatment with oxytetracycline, there was no statistically significant difference between the patients who received the antibiotic and those who received placebo capsules in respect to the incidence of exacerbations and the duration as judged by time lost from work. It was the authors' impression, nevertheless, that oxytetracycline therapy was of value. Edwards and his co-workers [*30*], in a study of fifty-three patients, came to a similar conclusion but supported it with statistically significant clinical evidence. They, however, noted no significant change in bacterial flora.

Douglas and his associates [*41*], in the same year at Edinburgh, studied the response to antibiotic therapy of 131 patients, eighty-nine with chronic bronchitis, during acute exacerbations. They concluded that routine bacteriologic examination of the sputum was of no value in predicting the response to treatment with any of the three drugs they used (penicillin, chloramphenicol or oxytetracycline).

Recently Cherniak et al. [*42*] in a long-term antibiotic double-blind study of sixty-seven patients with chronic bronchitis or bronchiectasis found that patients from whom H. influenzae was isolated had more days of lower respiratory tract infection than did those from whom the microorganism was never recovered. They observed no significant improvement in pulmonary function in the patients treated with antibiotics. However, patients who received tetracycline therapy had significantly fewer illnesses of the lower respiratory tract and these were of shorter duration than those in patients treated with placebos. The administration of tetracycline strikingly decreased the frequency of isolation of pneumococcus, but reduced the incidence of H. influenzae isolations insignificantly.

In a follow-up report of the microbial flora in this group of patients, subsequently expanded to include eighty-nine patients, Dowling and his associates [*43*] considered H. influenzae, Staph. aureus, and pneumococcus to be the most important pathogens and noted that "tetracycline or oleandomycin-penicillin therapy caused a significant diminution in the frequency with which these microorganisms were cultured from the sputum."

In a twelve-week investigation of the effects of erythromycin chemoprophylaxis in twenty patients with chronic obstructive pulmonary emphysema, Hallett, Beall and Kirby [*44*], employing a double-blind technic, found no significant difference between the patients treated with the drug and those treated with the placebo, except for the elimination of pneumococcus from the sputum of those receiving the antibiotic, an effect which was not correlated with subjective or objective clinical changes in the disease. They made no particular effort to isolate H. influenzae by using selective media.

More recently, Murdoch et al. [*34*] in Edinburgh reported that continuous tetracycline or oxytetracycline therapy during six winter months had a statistically significant beneficial effect. Patients who received antibiotic therapy had few or no sputum specimens containing pneumococcus or H. influenzae, were subjectively better, and lost fewer days from work than those receiving placebo therapy.

In summary, previous work has suggested several major conclusions. Infection has been considered important in the progression of chronic bronchitis. Not only the presence of pus containing neutrophils in the sputum of patients with chronic bronchitis, but also inflammatory changes in histologic sections have been interpreted to substantiate this belief. Although viral etiologies have been suggested, the infection in bronchitis has generally been thought to be bacterial. The correlation of pus with certain bacteria and the apparent response of some of these infections to the administration of antibiotics has supported this conclusion. H. influenzae and pneumococcus have been considered the main bacterial offenders because of their high incidence in cases of chronic bronchitis, their correlation with pus and their correlation with symptoms. There have, however, been few long-term serial observations on sputum to relate the flora to the development of acute exacerbations. Long-term antimicrobial therapy has been found to be of value by some, but not by others, and its effect on the sputum flora has not always correlated with clinical effects.

PLAN OF STUDY

Subjects. Twenty-nine patients were selected from the Emphysema Section of the Bellevue Hospital Chest Clinic. All attended regularly and had clinical and physiologic evidence of chronic bronchial obstruction and emphysema. Shortness of breath, wheezing, cough and sputum production were observed in all. Physiologic studies always showed an increased residual volume, an increased residual volume to total lung capacity ratio and reduced

maximum breathing capacity. The patients ranged in age from forty-five to seventy-six years, the mean being sixty-one. Twenty-seven were men, all but four of whom were too incapacitated by breathlessness to maintain a job. The two women were able to perform only limited housework. All had had pneumonia in the past or previous attacks of acute bronchitis. Although all the patients had had wheezing, none was considered (based on past history) to have true bronchial asthma. No systematic search was made for bronchiectasis, but good evidence that it was present was found in only three patients. Three patients had had cor pulmonale with congestive heart failure in the past and three additional patients were showing signs of incipient cor pulmonale when the study was undertaken.

Methods. The twenty-nine subjects were randomly assigned to two groups, one of which was treated with tetracycline,* 0.5 gm. given four times daily for forty-eight hours each week. The other group received a similar number of placebo capsules. The investigation was carried out between September 1956 and February 1958. All patients were followed up for from eleven to fourteen months of treatment.

Before treatment was initiated, each subject was required to submit a specimen of sputum at weekly intervals for at least three consecutive weeks. The patients were instructed to collect all the sputum they could produce with deep coughing from the time they arose in the morning until they came to the clinic a few hours later. This technic of sputum collection was maintained during the period of treatment. While there is controversy [*12,14–17,26*] over the question of whether sputum flora is truly representative of the bacterial flora of the bronchial tree, because sputum expectoration seemed to be the only practical method of collecting a large number of specimens over a prolonged period, it was the one chosen.

Throughout the study nearly all subjects were interviewed briefly each week when they submitted a sputum specimen and received their weekly supply of medicine. At monthly intervals, or more frequently in case of illness, patients were seen by clinic physicians who did not know in which group each of their patients had been placed. Patients were examined and questioned in detail to elicit any evidence of respiratory infection, additional antibiotic therapy they may have received from outside sources, failure to take the prescribed capsules or any possible toxicity from them during the preceding month. Additional antimicrobial therapy, usually with chloramphenicol, was permitted if an apparent infection seemed sufficiently severe. All patients in both groups received treatment with various bronchodilators. Digitalis preparations, diuretics and treatment with intermittent positive pressure respirators were used when appropriate.

* Tetracycline and tetracycline placebo capsules were supplied by Chas. Pfizer and Co., Inc.

The acute episodes that occur during the course of chronic bronchitis and emphysema are difficult to define, and precise criteria for assessing their duration and severity have not been developed. However, for this study an arbitrary definition of infection of the lower respiratory tract that referred only to clinical evidence and did not use bacteriologic findings had to be evolved. The following criteria were chosen: (1) a change on roentgenograms compatible with bronchopneumonia, or (2) acute worsening of the respiratory status accompanied by an increase in cough and at least one of the following: (a) a change in sputum to a more purulent character, (b) an increase in amount or change in viscosity of sputum together with one or more of these: fever, shaking chills, chest pain, increase in old or development of new localized physical signs, leukocytosis.

Vital capacity, functional residual capacity, maximum breathing capacity and arterial blood gas determinations were measured three to six months before and nine to eleven months after the beginning of therapy by the methods described by Baldwin, Cournand and Richards [*45*].

Specimens were routinely collected in sterile jars. All specimens were processed within six hours of collection. The quantity of each specimen was measured and the character described. For the first six months, sputum smears were stained with Wright's stain and for a shorter period with a special eosinophil stain described by Mulder [*18*].

Microscopic examination for cell types was made. During the first four months of the study, pancreatin digestion according to the method of Rawlins [*22*] was carried out before samples were taken for culture or smeared for cell types. Subsequently, all specimens of sputum were homogenized in a Virtis homogenizer until the organisms appeared on microscopic slides to be evenly distributed.

After homogenization, samples were serially diluted in broth and then inoculated onto blood agar and Levinthal plates. Samples were inoculated undiluted and at concentrations of 1:10, 1:100, 1:1,000 and 1:10,000. Undiluted samples were also routinely inoculated onto MacConkey plates, except during the early part of the study. All cultures were incubated at 37°c. and examined after twenty-four hours and again at forty-eight hours. Anaerobic cultures were performed during the first six months of the study, using thioglycollate broth or Brewer's plate. After the technic of homogenization was substituted for pancreatin digestion, samples for anaerobic culture were always taken from the most purulent portion of the specimen before it was homogenized. Whenever gram stain of the sputum or inspection of the blood agar plate suggested presence of fungi, Sabouraud's medium was also inoculated and incubated at room temperature.

The organisms were identified and the number of colonies of each species on a given plate were estimated. Bile solubility tests were carried out on all

suspected pneumococci and capsular typing was regularly performed. Coagulase testing was carried out on all staphylococci. Although such common microorganisms as N. catarrhalis, Strep. viridans, Staph. albus and diphtheroids were identified, they will not be mentioned further as previous studies have minimized their pathogenicity. Hemophilus was grown by the technic of Alexander [*46*] and H. influenzae distinguished from parainfluenza and H. hemolyticus. Capsular typing with a–f antiserum was attempted. Gram-negative enteric rods were usually further identified by growth in the appropriate media.

Sensitivity tests to tetracycline and chloramphenicol were carried out periodically by the tube dilution method on H. influenzae, Staph. aureus and gram-negative enteric organisms.

TABLE I

SUBJECTIVE REACTIONS OF PATIENTS RECEIVING THE PLACEBO AND PATIENTS RECEIVING TETRACYCLINE DURING THE TREATMENT PERIOD

A. General Subjective Response

Reaction	Patients Receiving the Placebo				Patients Receiving Tetracycline			
	At 6 mo.		At 12 mo.		At 6 mo.		At 12 mo.	
	No.	%	No.	%	No.	%	No.	%
Better	8	61.5	6	46.2	11	68.8	11	68.8
Unchanged	3	23.1	5	38.4	3	18.7	1	16.2
Worse	2	15.4	2	15.4	2	12.5	4	25

B. Reactions in Respect to Specific Symptoms

Reaction	Patients Receiving the Placebo			Patients Receiving Tetracycline		
	Cough	Sputum	Dyspnea	Cough	Sputum	Dyspnea
Better	4	4	4	6	5	7
Unchanged	7	8	7	4	7	5
Worse	2	1	2	6	4	4

RESULTS

Subjective Reactions. After six and twelve months of therapy each patient was asked whether he felt the same, better or worse than in the previous year and in what way he felt better or worse, with special reference being made to cough, sputum and dyspnea. (Table I.) At six months the reactions were remarkably similar in the two groups, but at the time of the later interview 46 per cent of the thirteen patients taking the placebo felt better while 69 per cent of the sixteen patients receiving tetracycline thought they were improved. At the same time, a greater number of the patients receiving tetracycline (25 per cent) thought they were worse.

When asked what seemed to help them most, the majority cited a variety of factors among which the "capsules" were usually not the most important. Cessation of smoking, the weather and use of an intermittent positive pressure breathing apparatus were among the more important factors considered to be influential.

No definite toxic reactions to tetracycline therapy were encountered. Four of the sixteen patients had mild symptoms which could be considered side reactions: three had transient diarrhea and one had substernal burning and belching on the days he took his capsules. One patient voluntarily discontinued medication after eleven months because of anorexia and a skin rash which was never observed by a physician.

Number of Infections. The peaks of infection occurred in January and in September and October 1957 and could be better correlated with the peak incidence of the Asian influenza epidemic in Manhattan than with the incidence of H. influenzae or of pneumococcus in the sputum. (Fig. 1.)

During the experimental period there were thirty-five infections of the lower respiratory tract in the group receiving the placebo compared to twenty-nine infections in the group treated with tetracycline. (Table II.) At the 0.05 significance level this difference is probably statistically significant. Forty-nine per cent of the infections in the patients receiving the placebo required treatment with an antibiotic, while 63 per cent of those in the patients receiving tetracycline were treated with an additional antibiotic. The percentage of infections requiring hospitalization, however, was comparable in the two groups, 23 and 24 per cent.

Sputum Character and Flora. Because the interval during which each patient collected his sputum specimens was relatively uniform, the volume of these specimens provides a rough quantitative estimate of the sputum production for each patient. Throughout the study, the volume of specimens submitted, except for mild fluctuations from week to week, was essentially unchanged for most patients. Two patients in both groups had a decrease in sputum volume during therapy, while one patient in each group had an increase in volume.

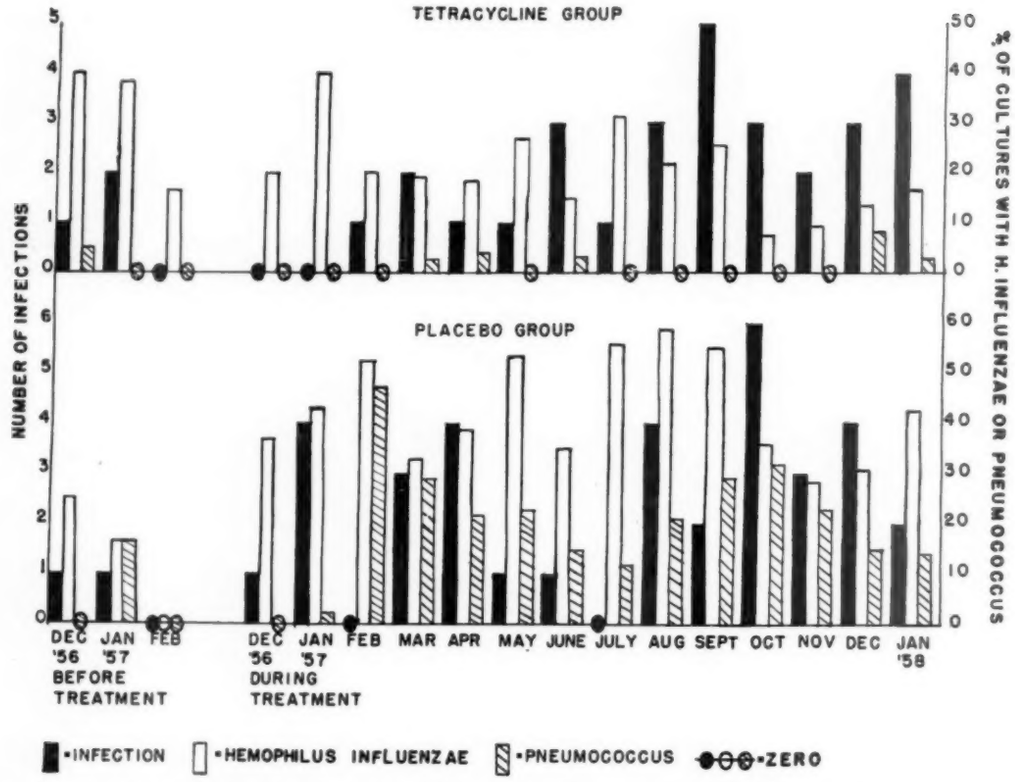

FIG. 1. Number of infections of lower respiratory tract and the percentage of sputum cultures containing H. influenzae or pneumococcus, by months, during the pretreatment and treatment periods. It should be noted that the number of patients under observation during the first three months of each of these periods was variable, as new patients were being introduced into the study. After March 1957 both groups were quite constant in composition.

Purulent sputum was considered to be sputum which was colored in shades of yellow, green or gray and was opaque or had clumps of opaque colored material in it. The presence of polymorphonuclear leukocytes in sputum with these gross characteristics was confirmed during the early part of this study by microscopic examination.

During the pretreatment period, twenty-five of the twenty-nine patients produced purulent sputum predominantly. After therapy, this number was essentially unchanged. (Table III.)

Approximately three-quarters of the specimens from each group of patients during the pretreatment period were purulent, whereas during the experimental period the incidence of purulent sputum rose slightly in the patients receiving the placebo but decreased in those receiving tetracycline. The difference in the per cent of purulent specimens in the two groups during therapy is significant with $P < 0.01$.

The pretreatment observations of sputum flora are shown in Table IV. Bacteriologically the two groups were not strictly comparable, there being a greater incidence of H. influenzae, Staph. aureus and gram-negative enteric microorganisms in the group treated with tetracycline. Although gram-negative enteric rods were not specifically cultured on MacConkey medium and individually identified during the early part of this study, they were present in 50 per cent of the patients during the pretreatment period and could not be correlated with prior antibiotic therapy. All but three of the total twenty-nine patients had received some antibiotic therapy within eighteen months of their first sputum observation, but the majority had received no antibiotic for at least three months before the first observation. There was no greater frequency of gram-negative enteric rods in the sputum of the six patients treated with antibiotics within one month of the study than in those treated more remotely in time; moreover, two patients with heavy persistent growth of these micro-

TABLE II

INCIDENCE AND SOME MEASURES OF SEVERITY OF CLINICAL INFECTIONS IN PATIENTS RECEIVING THE PLACEBO AND PATIENTS RECEIVING TETRACYCLINE DURING THE TREATMENT PERIOD

Data	Patients Receiving the Placebo	Patients Receiving Tetracycline
Total no. of infections	35	29
% of patients with infection	77	75
% of patients treated with another antibiotic	69	63
% of infections in which patients were treated with another antibiotic	49	62
% of patients hospitalized	46	31
% of infections in which patients were hospitalized	23	24
Days of hospitalization	214	254*
Days of other antibiotic therapy	280†	348‡

* One patient (H. M.) accounted for 149 days of hospitalization.

† Eighteen days of therapy were for non-respiratory infection (e.g., genitourinary infection).

‡ One patient (H. M.) accounted for 108 days of drug therapy.

TABLE III

SPUTUM PURULENCE BEFORE AND DURING TREATMENT

Data	Patients Receiving the Placebo	Patients Receiving Tetracycline
A. Pre-Treatment Period		
Total no. of patients	13	16
No. of patients with predominantly purulent sputum	12	13
Total no. of specimens	71	100
Purulent	55 (77.4%)	76 (76%)
Non-purulent	15 (21.1%)	24 (24%)
Not described	1 (1.5%)	0
B. Treatment Period		
Total no. of patients	13	16
No. of patients with predominantly purulent sputum	12	12
Total no. of specimens	346	432
Purulent	289 (83.5%)	289 (66.9%)
Non-purulent	57 (16.5%)	140 (32.4%)
Not described	0	3 (0.7%)

organisms had had no antibiotic therapy for more than eighteen months.

During the treatment period an immediate change in flora in the first specimen after therapy was started was considered to have occurred in only two patients. These patients had repeatedly had H. influenzae on the pretreatment cultures but had none on the first and subsequent cultures after initiation of treatment. The frequency of microorganisms in most patients was so sporadic during the pretreatment period that their appearance or disappearance immediately after treatment could not be judged as due to drug effect.

The incidence of possibly pathogenic microorganisms observed most frequently during the treatment period is shown in Table v. H. influenzae was isolated less than half as frequently from the patients treated with tetracycline as from those receiving the placebo, despite the fact that before therapy it was twice as prevalent in the tetracycline group. The frequency of H. influenzae isolations did not follow any seasonal pattern (Fig. 1), nor was its absence always correlated with antibiotic therapy. In some patients it was noted to come and go spontaneously without relation to therapy. H. influenzae isolated during the experimental period from the sputum of patients from both groups showed no consistent resistance. The majority of strains were sensitive to 6 μg. per cc. of tetracycline (occasionally 25 to 50 μg. per cc.) and to less than 6 μg. per cc. of chloramphenicol. Typing of H. influenzae revealed no encapsulated microorganisms in any patient.

The incidence of pneumococcus rose in the patients receiving the placebo from 4.2 per cent during the pretreatment period to 23.1 per cent of the total number of cultures during the experimental period. In the patients receiving tetracycline, on the other hand, the frequency of pneumococcus isolations fell to an almost negligible figure of 2.1 per cent. The seasonal incidence of pneumococcus is shown in Figure 1. All the pneumococci belonged to the higher types (VI to XXIX) or were non-typable.

Both H. influenzae and pneumococcus were found more frequently in purulent than in non-purulent sputum, but although the incidence of these microorganisms decreased significantly

TABLE IV

SPUTUM FLORA DURING THE PRETREATMENT PERIOD

A. Incidence by Cultures of Common Sputum Organisms

Organism and Incidence	Patients Receiving the Placebo	Patients Receiving Tetracycline
Duration of pretreatment period (patient-mo.)	32	36
Total no. of cultures	71	100
Hemophilus influenzae—total incidence (% of cultures)*	23.9	38.0
Incidence in purulent sputum	27.2	46.0
Incidence in non-purulent sputum	6.6	12.5
Pneumococcus—total incidence (% of cultures)†	4.2	4.0
Incidence in purulent sputum	3.6	3.9
Incidence in non-purulent sputum	0	4.2
Staphylococcus aureus—total incidence (% of cultures)	7.0	21.0
Incidence in purulent sputum	5.5	9.0
Incidence in non-purulent sputum	3.6	10.0
Gram-negative enteric microorganisms—total (% of cultures)	7.0	17.0
Incidence in purulent sputum	7.3	15.7
Incidence in non-purulent sputum	6.6	20.8

B. Incidence by Patients of Common Sputum Organisms

Organism	Patients Receiving the Placebo		Patients Receiving Tetracycline	
	No.	%	No.	%
Total	13		16	
Hemophilus influenzae	8	61.5	13	81.3
Pneumococcus	3	23.1	2	12.5
Staphylococcus aureus	4	30.8	7	43.8
Gram-negative enteric rods	6	50.0	8	50.0

* One specimen from a patient receiving the placebo was not described but contained H. influenzae.

† One specimen from a patient receiving the placebo was not described but contained pneumococcus.

TABLE V

SPUTUM FLORA DURING THE TREATMENT PERIOD

A. Incidence by Cultures of Common Sputum Organisms

Organism and Incidence	Patients Receiving the Placebo	Patients Receiving Tetracycline
Duration of treatment period (patient-mo.)	172	207
Total no. of cultures	346	432
Hemophilus influenzae—total incidence (% of cultures)*	46.8	18.8
Incidence in purulent sputum	51.9	23.2
Incidence in non-purulent sputum	21.1	7.9
Pneumococcus—total incidence (% of cultures)†	23.1	2.1
Incidence in purulent sputum	26.0	2.4
Incidence in non-purulent sputum	8.8	1.4
Staphylococcus aureus—total incidence (% of cultures)	23.4	17.8
Incidence in purulent sputum	22.8	17.3
Incidence in non-purulent sputum	24.6	18.6
Gram-negative enteric rods—total incidence (% of cultures)	48.8	53.9
Incidence in purulent sputum	49.1	57.8
Incidence in non-purulent sputum	47.4	47.1

B. Incidence by Patients of Common Sputum Organisms

Organism	Patients Receiving the Placebo		Patients Receiving Tetracycline	
	No.	%	No.	%
Total	13		16	
Hemophilus influenzae	12	92.3	14	87.5
Pneumococcus	12	92.3	7	43.8
Staphylococcus aureus	13	100.0	16	100.0
Gram negative enteric rods	12	92.3	16	100.0

* Three specimens from patients receiving tetracycline were not described, but contained H. influenzae.

† One of these specimens which were not described contained pneumococcus.

with antibiotic therapy, the majority of specimens during therapy remained purulent. (Tables III and vA.)

Staphylococci increased in the group receiving the placebo to approximate the pre- and post-treatment prevalence of 21 and 18 per cent in the group receiving tetracycline. Although every patient in both groups had coagulase positive Staph. aureus in at least one culture, at no time did overt staphylococcal infection become a problem. One patient treated with tetracycline was found to have a pure culture of Staph. aureus in his sputum on one occasion after hospitalization. Although he received no additional therapy, in subsequent sputum examinations the organism was present only sporadically,

as it had been originally. Drug sensitivities of microorganisms isolated from individual patients varied from week to week. Occasional resistance to tetracycline was noted before therapy, and the incidence of resistant staphylococci was not found to have increased by the end of the study. Staphylococci were found with approximately the same frequency in both purulent and non-purulent sputum. (Table vA.)

During the treatment period gram-negative enteric microorganisms were present in all but one of the patients receiving the placebo (Table vB), but were considered to be increasing in numbers or frequency towards the end of the study in only three patients, in none of whom antibiotic administration could have played a role. All the patients treated with tetracycline had gram-negative enteric rods, but in only six patients (less than half) did they increase. Three of these had recently received additional antimicrobial therapy. As in the case of staphylococci, gram-negative microorganisms were found with similar frequency in both purulent and non-purulent sputum from patients before and during therapy. The increased incidence of these organisms in sputum cultures during the treatment period (Table vA) was similar in both groups and may be due partly to the duration of the experimental period and to the more intensive search for gram-negative enteric rods as the study progressed.

Anaerobic cultures revealed an occasional anaerobic streptococcus but no bacteroides.

Although a variety of yeasts were found, Candida albicans was isolated in only one patient and that after a period of hospitalization with intensive additional antimicrobial therapy.

Correlation of Sputum Flora with Infection. The sputum flora at the time of infection may be divided into two categories: (1) that which appeared suddenly within two weeks of clinical infection, not having been previously repeatedly observed; and (2) that which was present at the time of infection, but which had also always been repeatedly observed in the same patient for weeks before and after infection without correlated clinical changes (termed "persistent" flora).

In Table vi are shown the microorganisms which first appeared within two weeks of clinical infection. The majority of infections, 79.3 per cent of those in the group receiving tetracycline and 68.6 per cent of those in the group receiving the placebo, were not associated with any immediate change in bacterial flora. Pneumococcus was the most frequently correlated species, being associated with nine infections in the group receiving the placebo and five infections in the patients treated with tetracycline. In one of the latter patients, there had been a lapse in medication for the previous three weeks. H. influenzae was never observed to appear with clinical infection and to disappear with subsidence of symptoms, or to increase in numbers at the time of acute episodes.

TABLE VI
CORRELATION OF SPUTUM FLORA WITH INFECTION*

Organism	Patients Receiving the Placebo, No. of Infections	Patients Receiving Tetracycline, No. of Infections
Pneumococcus	9	5†
Pseudomonas pyocyaneus plus Klebsiella aerogenes	0	1
Beta hemolytic streptococcus	1	0
Klebsiella aerogenes	1	0
Incomplete observations	1	1
No change in flora	23	22
Total no. of infections	35	29
% of infections associated with no change in flora	68.6	79.3

* Organisms which suddenly appeared, within two weeks of infection, not having been previously repeatedly observed.

† One of these infections occurred during a lapse in drug therapy for the previous three weeks in one of these patients.

In Table vii are shown the various combinations of "persistent" organisms which were present at the time of infection. H. influenzae was present in 52.9 per cent of the infections in the group receiving the placebo and in 37.9 per cent of the infections in the group receiving tetracycline, while gram-negative enteric rods were present in 50 per cent of the infections in the former group and in 58.6 per cent of the latter group.

Pulmonary Function. Results of pulmonary function tests are shown in Table viii. Vital capacity, functional residual capacity, maximum breathing capacity and arterial blood gases did not change significantly, as evaluated by the

TABLE VII
CORRELATION OF PERSISTENT SPUTUM FLORA WITH INFECTION *

Persistent Organisms	Patients Receiving the Placebo, No. of Infections	Patients Receiving Tetracycline, No. of Infections
H. influenzae:		
Heavy	2	3
Light or moderate	6	3
H. influenzae plus pneumococcus	3	0
H. influenzae plus gram-negative enteric rods	7	4
H. influenzae plus Staph. aureus	0	1
H. influenzae plus pneumococcus, plus gram-negative enteric rods	1	0
Gram-negative enteric rods:		
Heavy	3	7
Light	6	5
Gram negative enteric rods plus Beta hemolytic streptococcus	0	1
Staph. aureus	2	1
Staph. aureus plus gram-negative enteric rods	1	0
Non-pathogenic organisms	3	4
Incomplete observations	1	0
Total no. of infections	35	29
% of infections with persistent flora of H. influenzae	52.9	37.9
% of infections with persistent gram-negative enteric rods	50.0	58.6

* Organisms present during infections, but repeatedly observed before or afterwards in the same patient without clinical infection.

TABLE VIII
RESULTS OF PULMONARY FUNCTION TESTS

Lung Volumes (% of predicted value)	Patients Receiving the Placebo		Patients Receiving Tetracycline	
	Pre-treatment (mean)	Post-treatment (mean)	Pre-treatment (mean)	Post-treatment (mean)
Vital capacity	63.1	61.3	62.5	56.3
Residual volume	258.6	234.0	239.4	218.0
Total lung capacity	122.9	119.0	114.8	106.5
Residual volume/total lung capacity × 100	61.4	58.7	61.8	58.3
Maximum breathing capacity (% of predicted value)	39.6	33.1	38.7	34.3
After bronchodilator	42.4	37.6	44.3	43.2
Arterial blood O_2 saturation (%)	89.1	90.5	90.6	90.0
pCO_2 (mm. Hg)	45.5	48.2	43.8	45.5

t test when each group was compared with itself or with each other.

COMMENTS

While the present study confirms many of the earlier observations that have been made on the bacterial flora and the effects of chemoprophylaxis in patients with chronic bronchitis and emphysema, it has also led to some conclusions that are quite different. An examination of these similarities and differences is important.

Pneumococcus and H. influenzae, as in most other series, were commonly found in the sputum. The incidence of H. influenzae in this series of patients is very similar to that published by Mulder et al. [*19*] and May [*36*] in cases of bronchitis and by Franklin and Garrod [*47*] in cases of bronchiectasis in children. Their findings of H. influenzae in 80 to 90 per cent of patients with mucopurulent or purulent sputum or with bronchiectasis compares well with the finding of it in 92 per cent of the patients receiving the placebo in this study during an eleven to fourteen month period. However, the presence at some time of pneumococci in 92 per cent and staphylococci in 100 per cent of these patients, although different from the incidence of these microorganisms reported by May and Oswald [*39*] and May [*23,36*], was not unexpected. Undoubtedly the greater number of cultures in the present series, collected repeatedly in the same patients over a longer period of time, enhanced the chances of isolating these microorganisms. The incidence of a microorganism is probably better expressed as a per cent of the total number of *cultures*, rather than as the per cent of patients having the microorganism at some time, as Straker, Hill and Lovell [*48*] have shown, for example, that if repeated samples are taken over a long enough period of time almost every subject will be found to harbor pneumococcus at one time or another.

When the incidence is expressed in per cent of cultures, the results of the present study are similar to those of May [*23*], May and Oswald [*39*] and Mulder and his co-workers [*19*] in respect to pneumococcus and Staph. aureus, but the frequency with which H. influenzae was isolated is a little less than in the series of May [*36*], Elmes et al. [*40*] and Edwards and his

associates [*30*], and comparable to that in the patients of Dowling and his co-workers [*43*]. Some variation is expected from series to series because of differences in bacteriologic technic as well as possible differences in the subjects, and the duration of observations (e.g., many cultures over a long period of time, versus single specimens). The slightly lower incidence of hemophilus in this study cannot, however, be attributed to faulty bacteriologic technic as this microorganism was specifically sought for and found in considerable numbers.

In this study the majority of infections could not be correlated with any immediate change in flora, a finding similar to that of Edwards et al. [*30*], Stuart-Harris and his associates [*25*], Clifton and her co-workers [*27*], Knox, Elmes and Fletcher [*29*] and Douglas and his associates [*41*]. One of the criteria for determining whether a specific clinical infection could possibly be caused by a certain microorganism known to be a potential pathogen is the sudden appearance or increase in numbers of that microorganism at the time of clinical infection. In only twelve infections in patients receiving the placebo and six infections in those receiving tetracycline were these criteria met. Although with biweekly cultures the optimum time for isolating the pathogen might have been missed, freshly cultured sputum from acutely ill patients frequently showed no specific change in flora, nor any increase in numbers of existing potential pathogens. It therefore seems likely that the majority of acute episodes were not caused by bacteria which are usually considered pathogenic. Dingle and his associates [*49*], in a carefully conducted three-year study of common acute respiratory diseases in a group of Cleveland families, found that bacterial infections accounted for only 2.5 per cent of the total cases of respiratory illnesses. Although many investigators of chronic bronchitis mention the possibility of non-bacterial factors in the acute exacerbations, a number of them, including those who found no change in flora, assume a bacterial component in view of the occasional reports of an increase in pathogens (particularly H. influenzae and pneumococcus) at the time [*18,32,34,40*], the correlation of specific bacteria with pus in the sputum [*23,24, 28–30,36*] and the apparent response of these illnesses to antibiotic therapy [*28,29,39–41*].

The failure to observe a change in bacterial flora at the time of exacerbations has not only etiologic but also therapeutic implications. If the common acute infections of the respiratory tract, which evidence intimates are predominantly viral, are responsible for initiating these repeated acute episodes of bronchitis, then, as Knight and White [*50*] have suggested, any control of bronchitis in patients with chronic pulmonary emphysema will depend upon both the extent to which bacterial pathogens complicate the bronchitis and the degree to which bacterial infection responds to antimicrobial therapy. It is interesting that in the present study the group receiving the placebo exceeded the group receiving tetracycline by the number of infections which were associated with specific change in flora, a finding which implies that tetracycline therapy did prevent some of the few infections which were apparently bacterial in origin.

For the most part, however, this study along with reports by Hallett et al. [*44*], Elmes, Fletcher and Dutton [*40*] and Knox and her associates [*29*] suggests that chemoprophylaxis is of doubtful value, a conclusion differing from that of many authors [*19,21,34,37–39,42,43*]. Some of these contrasting views may result from differences in the patients studied and their environments, and the design of the clinical experiment. The use of a control group, the drug regimen employed, the definition of infection, and the duration of the observation period may all affect the final results.

While it is clear that all of this group had some chronic bronchitis, as defined by Oswald, Harold and Martin [*51*], most of the British groups did not have pulmonary function studies that permit one to evaluate the disability or measure the physiologic characteristics. It is apparent that the group reported here had more severe disability than the majority of patients reported by May and Oswald [*39*], Edwards and his associates [*30*], Elmes et al. [*40*], or Murdoch and his coworkers [*34*].

At the time this study was initiated the two drugs that were principally considered for a chemoprophylactic trial were tetracycline and chloramphenicol. They could both be taken orally, had a fairly wide antibacterial spectrum, and were known to be effective against pneumococcus and H. influenzae microorganisms which earlier studies had suggested might be important. Tetracycline was finally chosen because of the occasional reports of aplastic anemia in patients treated with chloramphenicol. A regimen employing tetracycline during a forty-eight-hour period each week was selected because it

would be economical and might reduce the risk of causing a harmful change in flora. A dosage of 2 gm. per day rather than 1 gm. was chosen because of the short period of administration.

Benefits from long-term chemoprophylaxis may be evaluated in respect to effects on the sputum flora, purulence of sputum, number of acute infections of the respiratory tract, patient's subjective reactions and pulmonary function as measured objectively. Many previous reports do not consider all of these categories in their over-all evaluation of the effectiveness of antibiotic prophylaxis.

Except for Edwards and his associates [*30*] most investigators, including ourselves, have found an alteration in flora with long-term chemoprophylaxis, particularly a decrease in frequency or absence of pneumococcus isolations, with a variably significant decrease in H. influenzae, compared with the incidence in control patients. The relationship of these bacterial changes to clinical status is not clear, however. A statistically significant beneficial effect has been reported clinically by both those who observed a significant reduction in pneumococcus with less reduction in the incidence of H. influenzae [*42*] and those who, in contrast, found little change in flora with long-term antibiotic therapy [*30*].

The majority of patients in this series had purulent sputum before therapy, and although the frequency of purulent samples was significantly reduced in the group receiving tetracycline during therapy, the majority of patients continued to have more purulent than nonpurulent specimens. Many of the reports dealing with antibiotic therapy or prophylaxis in chronic bronchitis do not give specific details about the effect of drugs on purulence; some state that with short courses of antibiotics, purulence was abolished or lessened, only to return shortly after therapy was stopped [*23,28*]. However, using continuous tetracycline or oxytetracycline in doses of 1 to 1.5 gm. daily for six months, May and Oswald [*39*] reported a more striking conversion by gross inspection of purulent to mucoid sputum than was found in the present study. No detailed bacteriologic data are given, but reduction in purulence might be considered to be due to decrease in incidence of pneumococcus and H. influenzae in the treated patients. In the present study, however, as pneumococcus was almost abolished and H. influenzae was reduced to half its former incidence without a comparable decrease in purulence, it seems that these two organisms were not entirely or even mainly responsible for that gross characteristic of sputum.

Although the incidence of acute infections of the respiratory tract was reduced in the patients treated with tetracycline, there were so many episodes in both groups of patients that chemoprophylaxis must be considered to have had only a minor effect. Hallett et al. [*44*] in a shorter term experiment found no statistically significant difference in the number of infections in those receiving the drug and those receiving the placebo. Most other investigators have not given specific information about the incidence of acute episodes when a prolonged continuous chemoprophylactic agent was used. May and Oswald [*39*] mention that the incidence of these illnesses is decreased but do not give statistical data with simultaneous control groups. Although Edwards et al. [*30*] include this entity in their statistical analysis, it is difficult from their report to tell whether by itself the difference in incidence of infections in their two groups is significant. McVay and Sprunt [*37*] Cherniak et al. [*42*] and Murdoch and his associates [*34*] are among the few who specifically state that the frequency of acute infections of the respiratory tract is significantly reduced in patients treated chemoprophylactically.

Mulder [*19*], Helm et al. [*38*] and Oswald [*39*] have particularly cited the patients' subjective reactions to their medications as evidence of beneficial effects of chemoprophylaxis, and several studies employing control subjects [*34,37,42*] have supported these conclusions. However, in the present series the difference in reactions between those receiving the drug and those receiving the placebo is not very striking, and diminished with time.

The finding of no significant change in the results of pulmonary function tests in the two groups was to be expected, as they are rather gross tests, and it supported the observations of others [*42,44*] that chemoprophylaxis does not appear to alter pulmonary function.

Regardless of the somewhat dubious beneficial effects of chemoprophylaxis, this study along with others [*34,37–39,43*] suggests that there are no significantly harmful results. Unlike the group of unconscious patients treated by Petersdorf and his associates [*52*], the present group treated with tetracycline did not have more secondary bacterial infections than the group

treated with the placebo. In respect to the low incidence of side reactions and the failure to find a harmful alteration of sputum flora with resistant H. influenzae or predominance of gram-negative microorganisms or resistant staphylococci, the present study confirms the observations of others [*34,37–39,43,44*].

H. influenzae is considered by Mulder and his co-workers [*19,20*] and by May and Helm [*21,23,24,36*] and their associates, as well as by Elmes, Knox and Fletcher [*28*] and by Edwards et al. [*30*], to be particularly important in causing the repeated infections in patients with chronic bronchitis for several reasons, but in particular because of its high incidence in this group of patients [*19,21,23,39*]. Although the present study confirmed this observation, it is important to note that in no case did H. influenzae satisfy the criterion for significant relationship to infection by sudden appearance or increase in numbers at the onset of clinical infection. Despite the fact that it was frequently present during infection it had always been repeatedly observed in the same patient for weeks before and after infection without correlated clinical changes. Similar failure to observe significant proliferation of H. influenzae with exacerbations was also reported by Knox et al. [*29*] who concluded that although this microorganism was important in chronic bronchitis, its exact role is not clear. Glynn's [*53*] failure to find a close correlation of H. influenzae antibody titers with active infection provides additional evidence that H. influenzae is probably not responsible for the acute infections. Moreover, in the present study, patients with heavy persistent growth of H. influenzae did not tend to have more infections than those without heavy persistent growth in contrast to the observations of Knox, Elmes and Fletcher [*29*] and Cherniak et al. [*42*]. Furthermore, elimination of H. influenzae in several patients was followed by continued deterioration. In these patients gram-negative enteric rods became predominant and may have played a role in the ultimate course. The only evidence, from our data, of a possible etiologic role of H. influenzae in infection is the observation that although it was found in only 19 per cent of all cultures from patients receiving tetracycline, it was present in 38 per cent of the cases of clinical infection in that group. However, the hemophilus organisms were non-encapsulated, i.e., resembled those found in the healthy human nasopharynx. Alexander [*46*] states, on the basis of her own observations and those of others, that "the pathogenic potentialities of H. influenzae for humans is closely related to the presence of a capsule and the elaboration of a specific soluble substance. . . . Non-encapsulated H. influenzae seldom invades the blood and it is seldom possible to assign a primary pathogenic role to non-encapsulated H. influenzae in any age group."

The second reason why the groups at the Brompton and Hammersmith Hospitals in London [*23,24,28*] and those at Leeds [*30*], at Edinburgh [*34*], and in Holland [*19*] consider H. influenzae a significant pathogen is its correlation with the purulence of sputum. The fact that H. influenzae is found more commonly in purulent than in mucoid sputum and that purulence tends to disappear when H. influenzae is reduced or eliminated has suggested to these investigators that it is a causative organism. In the present study the sputum often looked green in the presence of heavy growth of H. influenzae and became paler when this microorganism disappeared after antibiotic therapy; but H. influenzae in both small and large numbers was also found not infrequently in grossly non-purulent specimens. That H. influenzae is not always associated with purulence, a finding also of May [*36*], Elmes et al. [*28,40*] and Edwards and his co-workers [*30*], suggests that the mere presence of this microorganism does not necessarily signify its primary pathogenicity in cases of bronchitis.

Another reason for possibly believing that H. influenzae is a harmful microorganism in these patients is that patients reportedly feel subjectively better when it has been eliminated by antibiotic therapy [*19,39*]. In the present series of patients there seemed to be no close correlation between symptoms and the frequency of hemophilus in the sputum. Others [*28,30,38*] have also found lack of correlation between bacterial flora and clinical improvement; i.e., persistence of H. influenzae in the sputum was sometimes associated with marked clinical improvement during treatment with an antibiotic.

Furthermore, H. influenzae did not seem to affect pulmonary function as we could measure it. Although the group receiving tetracycline had about 50 per cent less H. influenzae in their sputum than the group receiving the placebo, there was no significant difference in pulmonary function between the groups or between the

patients who produced heavy growth of hemophilus and those who never had it.

CONCLUSIONS

Pneumococcus and H. influenzae occur frequently in the sputum of patients with chronic pulmonary emphysema.

Most acute infections are not due to identifiable bacterial pathogens.

A few acute exacerbations appear to be due to infection with pneumococcus and these may be prevented by chemoprophylaxis.

The regimen of intermittent tetracycline prophylaxis used in this study is otherwise of questionable value, because (1) while it resulted in a statistically significant reduction in the number of infections of the lower respiratory tract in the treated group, the infection rate was so high that the effect of prophylaxis as given here must be considered minimal; (2) subjective improvement was noted with only slightly greater frequency in the group receiving tetracycline than in the group receiving the placebo; (3) objective evaluation of pulmonary function revealed no significant change in either group during the period of study.

H. influenzae is of doubtful importance in causing the acute episodes seen repeatedly in these patients because (1) there was no relationship between the development of acute exacerbations and the new appearance of H. influenzae or increase in the number of these microorganisms in the sputum; (2) patients with persistent heavy growth of H. influenzae had no more acute exacerbations than those without H. influenzae; (3) reduction of H. influenzae by chemoprophylaxis was associated with no marked reduction in clinical evidence of infections; (4) patients with acute exacerbations had no greater frequency of H. influenzae than those who never had acute episodes during the period of study.

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A Non-Atheromatous Proliferative Vascular Lesion of the Retina in Diabetes Mellitus*

Role in the Etiology of Diabetic Retinopathy

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THE increased frequency of the vascular complications of diabetes is often ascribed to improved control of carbohydrate utilization and ketosis, permitting diabetic patients to live long enough to have vascular disease. Emphasis with respect to these complications thus is now placed on the duration rather than on the severity of the diabetic state [*1,2*]. While this might account, in part, for the purported increase in the frequency of arteriosclerosis, there is some question as to whether or not increased longevity plays a significant role in those vascular phenomena which are considered to be an integral part of the disease. For, as Ashton [*3*] has pointed out, diabetic retinopathy "affects the eyes of young subjects as well as the old, the careful patient as well as the neglectful." The same statement can undoubtedly be made about nephropathy and, possibly, about some of the other vascular complications. Furthermore, there is suggestive evidence that these complications may occur before the onset of clinical manifestations of abnormal glucose utilization [*4–9*].

LeCompte [*10*] has pointed out that there are two separate vascular problems in diabetes. The first, arteriosclerosis, does not differ qualitatively in those with diabetes and those without, but appears to be of increased frequency and intensity in the diabetic subjects. The second vascular disease is believed to be an integral part of diabetes; LeCompte [*10*] has in fact suggested that the genetic fault responsible for diabetes may also be responsible for the related vascular phenomena. The occurrence of the latter in the retina and renal glomeruli have received considerable study and emphasis, but they also occur in nerves in which they may be responsible for diabetic neuropathy [*11,12*], and it has even been suggested that they may occur in the islets of Langerhans, in which they may be responsible for the hyalinization of the latter structures [*13*].

Some years ago Friedenwald [*14*] remarked that it indeed seemed strange that the vascular phenomena which appeared to be an integral part of the diabetic state should have only certain organs of predilection, and he prophesied that with careful search and improved technics they would be discovered in other sites. In the past several years we have attempted to determine whether or not Friedenwald's prophesy was correct. We have found a vascular lesion characterized by endothelial proliferation accompanied by the deposition of a network of fine fibrils which are periodic acid-Schiff-positive and unstained by colloidal iron, in contrast to the lesions of arterio- and arteriolosclerosis which usually vary in their reaction to periodic acid-Schiff staining, most often adsorb colloidal iron, and frequently show some degree of elastic fiber reduplication, which is not seen in the proliferative lesion. The arterio- and arteriolosclerotic lesion advances by the deposition of an acellular hyaline material, sometimes in association with lipid, whereas the proliferative lesion develops primarily as a result of an increase in endothelial cells and shows little or no lipid. These proliferative lesions have been seen in the placentas of

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diabetic mothers [*15*], in the vessels of the lower extremity of patients with diabetic gangrene [*12*], in the nutrient arteries to nerves of such extremities [*12*], and in the intramural branches of the myocardium [*16*]. While they do not occur exclusively in diabetes, their frequency is considerably greater in this disease than in a general autopsy population.

In the past two decades there has been considerable interest in the retinopathy of diabetes. Various studies have reported an incidence varying from as low as 4.1 per cent [*17*] to as high as 50 per cent [*18,19*]. As in the case of the other vascular complications of diabetes, the incidence seems to increase with increasing duration of the disease; this appears to be borne out by the older studies of Waite and Beetham [*20*] and the more recent ones of Becker [*19*], as well as those of Whittington and Lawrence [*18*] which show an incidence of about 75 per cent in patients with diabetes of fifteen to twenty-five years' duration. According to Becker [*19*] retinopathy is found in almost every patient with diabetic glomerulosclerosis, and Fagerberg [*11*] has reported that over 90 per cent of patients with diabetic neuropathy also have retinopathy.

The present report deals primarily with the occurrence of proliferative vascular lesions in the retina similar to those already described at other sites. While this lesion has been previously observed in the retina [*21*], it was considered by Ashton [*3*] to be secondary to the development of retinal microaneurysms. Histochemical procedures have been applied in this investigation which have not heretofore been utilized in the study of retinopathy, and which shed further light on the characteristics of this vascular disease. Finally, certain corollary observations have been made which suggest a possible etiology not heretofore considered.

MATERIAL AND METHOD

Eighty-four eyes were used in this study. Of these, thirty-four were from diabetic patients and fifty from non-diabetic subjects. Ten specimens were available as paraffin blocks;* the remainder were obtained at autopsy. In all but fifteen of the latter, the enucleated eye was fixed in 10 per cent formaldehyde, divided into eight sagittal rings, and the rings dehydrated and embedded in paraffin for section in the usual manner. In fifteen specimens only half of the eye (four rings) was so treated, and the other half was used for frozen section and fat staining.

Each paraffin block was sectioned at 6 mμ and four consecutive sections were stained with hematoxylin and eosin, the Verhoeff-van Gieson stain for elastic and other connective tissues, a modified periodic acid-Schiff technic, and the colloidal iron technic of Rinehart and Abul-Haj [*22*]. A fifth successive section was subjected to microincineration for the *in situ* study of calcium distribution. In selected eyes, sections were stained by the aldehyde fuchsin-alcian blue technic of Spicer and Meyer [*23*] and by the acid-fast technic for ceroid. In fifteen specimens, frozen sections were made and stained with oil red O for lipids.

As in previous studies dealing with diabetic gangrene [*12*] and myocardial infarction [*16*], these eye sections were studied and the results recorded without prior knowledge of the clinical and ophthalmoscopic findings. The retinal vessels were divided into two groups, those ranging up to approximately 30 μ in diameter (small), and those ranging over 30 μ (medium). Vascular lesions were then tabulated according to the following criteria:

Normal Arteries. Normal retinal arteries showed a distinct inner lining of fusiform endothelial cells in a single layer, beneath which there was a thin layer of muscle in which occasional nuclei could be identified. On periodic acid-Schiff (PAS) stain a distinct subendothelial basement membrane was seen which was characteristically PAS-positive. This basement membrane also gave a characteristic blue color with the colloidal iron technic, indicating that it contained sulfated mucopolysaccharide. The normal retinal arteries uniformly showed no evidence of elastic tissue and, except for slight accentuation of the endothelial surface, there was no evidence of significant calcium deposition.

Inflammatory Lesions. The criteria set up by Saphir et al. [*24*] and subsequently used by us [*16*] for the identification of such lesions in intramural coronary artery branches were also used here. Such lesions usually showed diffuse fibrocollagenous thickening of the artery wall, often accompanied by periadventitial fibrosis and lymphocytic infiltration. (Fig. 1.) Occasionally intimal fibrosis with luminal narrowing also was seen, and sometimes hemosiderin-containing macrophages were present. If in addition, endothelial proliferation was present, the specimens were also classified as in the proliferative group.

Thrombotic Lesions. Arteries or veins containing masses of fibrin, sometimes enmeshing erythrocytes, and often attached to the endothelial surface were placed in this group. (Fig. 2.) When, in addition, there was evidence of inflammatory reaction, they were also placed in the inflammatory group.

Atheromatous Lesions. Vessels taking the oil red O stain (Fig. 3) or showing large lipophage deposits were placed in this category.

* We are indebted to Col. Frank H. Townsend, Col. Joe M. Blumberg and Capt. Roger H. Fuller of the Armed Forces Institute of Pathology for providing these ten specimens.

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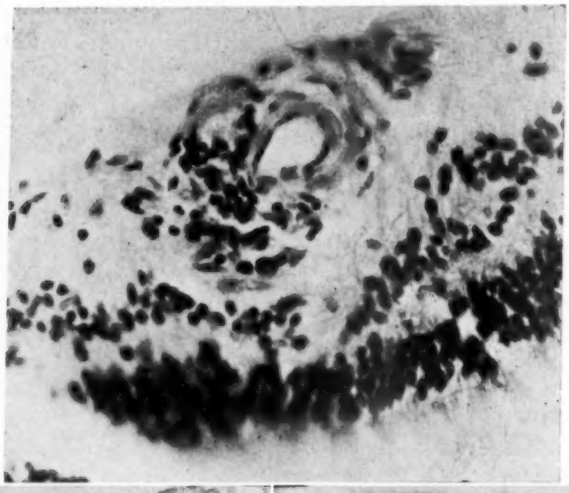

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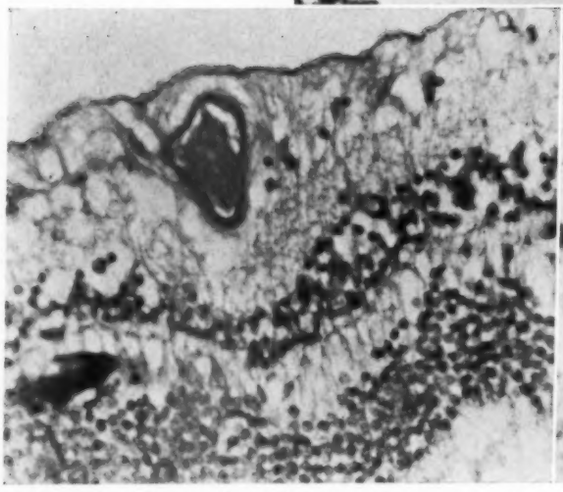

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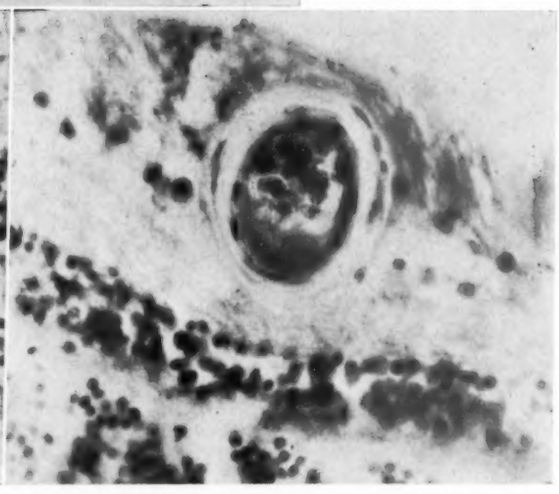

FIG. 1. Hematoxylin and eosin stain, original magnification × 120. Vessel shows a somewhat thickened fibrotic wall. Along the left side of the vessel there is an infiltration of lymphocytes and macrophages, the latter containing hemosiderin.

FIG. 2. Periodic acid-Schiff stain, original magnification × 120. Vessel is of normal thickness and contains a fibrin thrombus which shows only a faint PAS-reaction.

FIG. 3. Oil red O stain, original magnification × 250. The gray acellular material distributed irregularly along the inner portion of the wall gave an intense red reaction for lipid.

Hemodynamic Lesions. The earliest forms of this lesion in medium-sized arteries consisted of reduplications of the PAS-positive endothelial basement membrane. (Fig. 4.) With the colloidal iron stain such lesions showed an endothelial lining consisting only of a single layer of cells, beneath which there was a fairly thick subintima consisting of yellow-staining fibrocollagenous tissue containing focal amorphous deposits of a material taking colloidal iron. (Fig. 5.) In more advanced stages the fibrocollagenous layer became thicker and replaced muscle; it was PAS-negative, with only a thin inner rim of weakly PAS-positive material remaining (Fig. 6), and showed no evidence of elastic fiber formation. In small arteries and arterioles the hemodynamic lesion consisted of concentric rings of fibrocollagenous tissue which produced marked luminal narrowing, and only an occasional endothelial lining cell could be identified (Fig. 7); such lesions usually showed a slight increase in calcium.

Proliferative Lesions. In this group were placed those specimens in which the retinal arteries showed extensive endothelial proliferation. (Fig. 8 through 12.) Such changes were sometimes found in first or second order branches of the central artery (Fig. 10 and 11), but more often in small arteries. (Fig. 9.) They could be seen in longitudinal (Fig. 8 through 12) as well as in cross section. (Fig. 10 and 11.) While they were most numerous in the retinal arteries, they were occasionally encountered in other layers of the eye. Occasionally, vessels with eccentrically placed, round hyaline masses resembling the nodules of

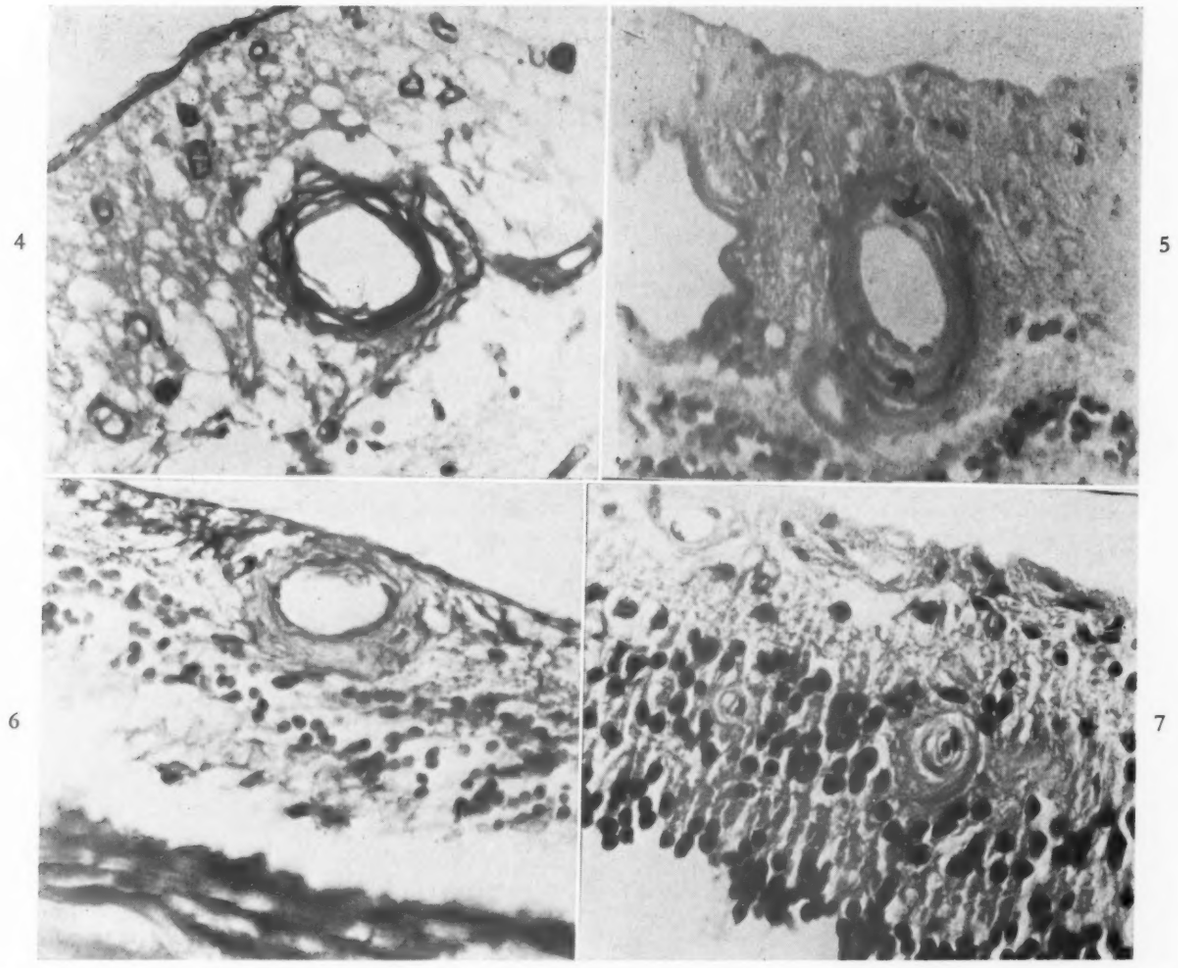

FIG. 4. Periodic acid-Schiff stain, original magnification × 250. The large artery in the center shows an early reaction consisting of splitting of the PAS-positive layer.

FIG. 5. Colloidal iron stain, original magnification × 250. The vessel along the left margin is a vein, the one in the center an artery. The latter is thickened, and the light gray areas (arrows) indicate foci of colloidal iron positive (blue-staining) material.

FIG. 6. Periodic acid-Schiff stain, original magnification × 250. The artery shows marked fibrous thickening and an almost complete loss of PAS-staining material.

FIG. 7. Hematoxylin and eosin stain, original magnification × 250. Section shows advanced lesion of small artery, with obliteration of lumen.

diabetic glomerulosclerosis were encountered. (Fig. 13.) These were rare, and since there was no direct proof that they represented an end stage of the proliferative lesion, they were not counted in this group.

The proliferative lesions appeared in their earliest stages as a prominent swelling of the endothelium (Fig. 8 and 9), followed by either concentric or eccentric proliferation of these cells to produce either progressive symmetrical closure of the lumens (Fig. 10 and 11) or eccentrically placed mounds. The endothelial proliferation was accompanied by deposition of a fine meshwork of PAS-positive fibrils which appeared to contain no sulfated mucopolysaccharides, so far as could be established by the colloidal iron and aldehyde fuchsin-alcian blue technics. They also gave a negative reaction for lipids, as well as for ceroid, and showed no significant increase in calcium.

In addition to tabulating vascular lesions, extravascular changes in the retina were also recorded. Particularly noted were exudates (Fig. 14) as well as various types of cellular reaction. (Fig. 15, 16 and 17.)

After all the data obtained from microscopic study had been recorded, correlations with clinical findings were made and tabulated, as shown in Tables I through IV. These included tabulation according to diabetic and non-diabetic categories, as well as according to age, sex and the presence or absence of

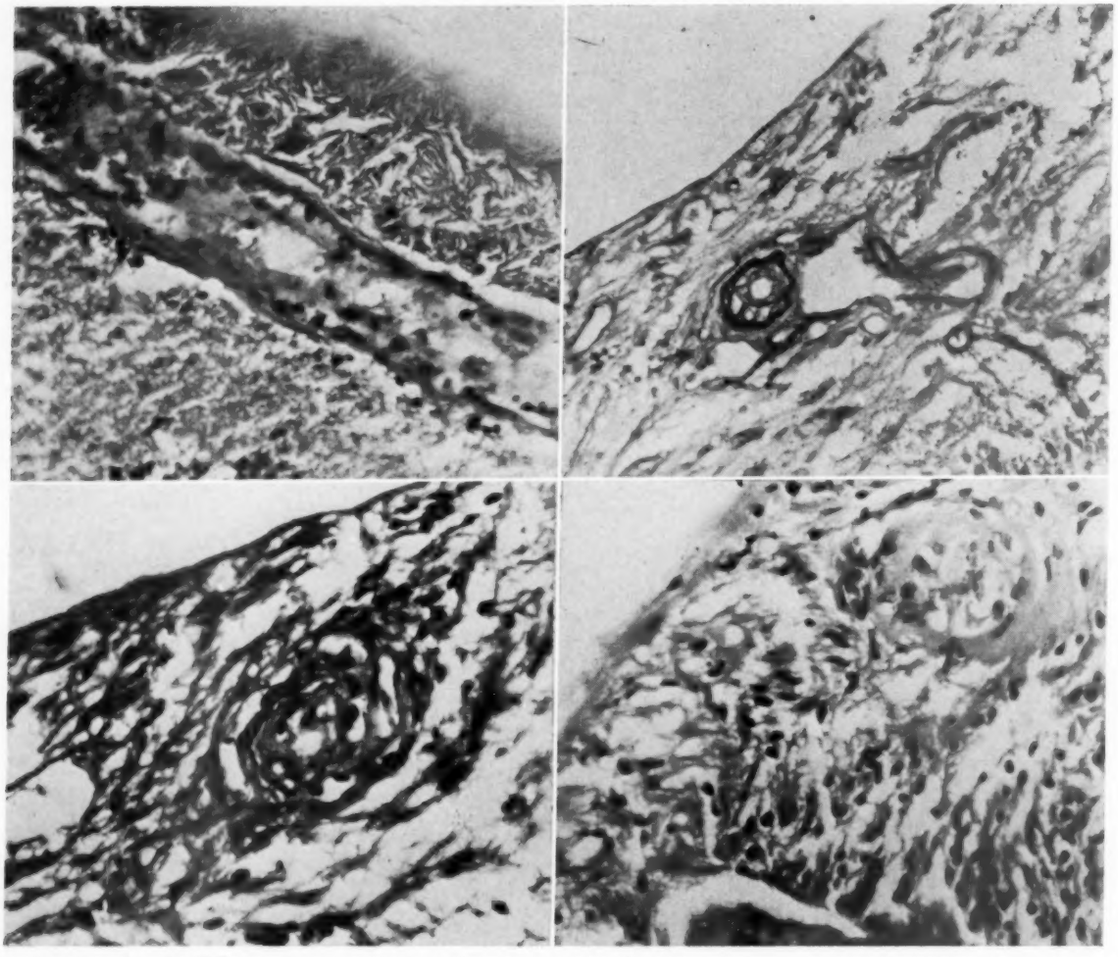

FIG. 8. Verhoeff-van Gieson stain, original magnification × 250. Section shows a vessel in longitudinal section. Note enlarged endothelial cells proliferating to form bridges across the lumen. Oil red O stain on this vessel was negative.

FIG. 9. Periodic acid-Schiff stain, original magnification × 250. Dilated thin-walled channels are veins. Vessel just to the left of center is an artery showing endothelial proliferation into lumen. Beneath the endothelial cells there is a reduplicated layer of PAS-positive material.

FIG. 10. Verhoeff-van Gieson stain, original magnification × 400. In the center there is an artery showing marked endothelial proliferation with marked narrowing of the lumen.

FIG. 11. Hematoxylin and eosin stain, original magnification × 400. At the top (arrow) there is an artery showing hyalinization of the wall and endothelial proliferation with complete obliteration of the lumen.

hypertension. Subjects were considered hypertensive when there was a history of persistent blood pressure in excess of 150/90 mm. Hg.

RESULTS

Population Characteristics. In Table 1 the subjects represented in this study are divided into diabetics and non-diabetics, and according to age (up to fifty and over fifty years), sex, and the presence or absence of hypertension. Five of the thirty-four diabetic patients (15 per cent) were in the age group up to fifty as compared to eleven of the fifty non-diabetic subjects (22 per cent). Twenty of the thirty-four diabetic patients (59 per cent) were males, as were twenty-nine of the fifty non-diabetic subjects (58 per cent). Twenty-eight of the thirty-four diabetic patients (82 per cent) had hypertension as compared to thirty-one of the fifty non-diabetic subjects (62 per cent). Thus the sex distribution in the two groups was similar but there were relatively more subjects with hypertension and relatively fewer subjects under age fifty among the diabetic patients. All the diabetic patients have received insulin.

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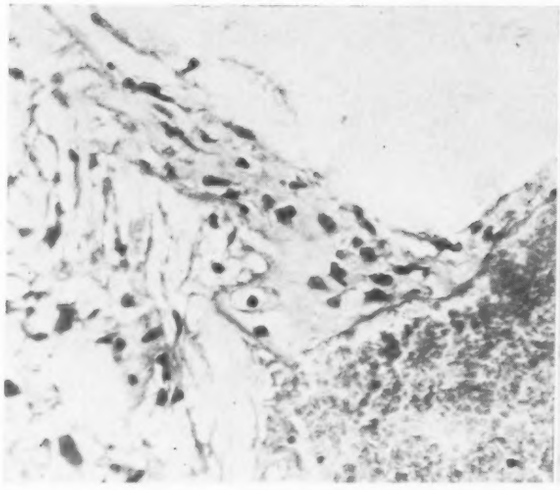

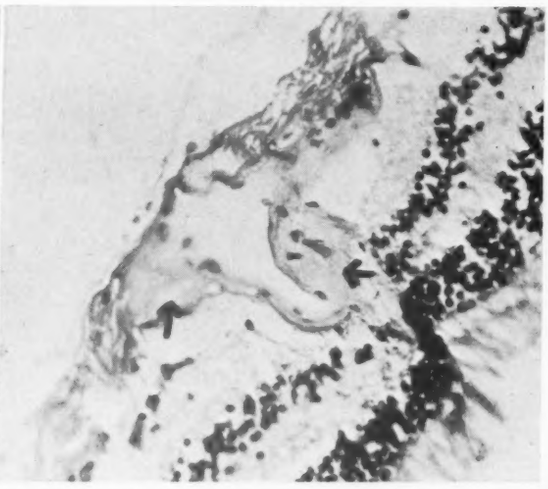

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FIG. 12. Verhoeff-van Gieson stain, original magnification × 400. Longitudinal section of vessel communicating with an aneurysm. The latter is at the lower right hand corner of the figure. Note swollen endothelial cells with obliteration of the lumen.

FIG. 13. Hematoxylin and eosin stain, original magnification × 250. This retinal vessel contains two hyaline masses (arrows) resembling nodular lesions of diabetic glomerulosclerosis.

Inflammatory Lesions. Inflammatory lesions were seen about arteries as well as veins, and in almost all the structures of the eye, most frequently in the conjunctiva. However, the data in Table II refer only to those specimens with lesions in the retina. Retinal lesions appeared with increased frequency among diabetic patients, occurring almost fifteen times more often than in the non-diabetic subjects. The frequency with which hemosiderin-containing macrophages were seen in such lesions suggests a greater frequency of retinal hemorrhages among diabetic patients, and that inflammatory vasculitis in diabetes often is a reaction to such hemorrhage.

Thrombotic Lesions. As shown in Table II, thrombotic lesions occurred rarely in both groups. While the percentage is higher among those with diabetes, the small numbers in both groups showing this lesion makes the significance of this difference doubtful.

Atheromatous Lesions. The blood retained in vessels in both groups often gave a positive oil red O reaction, and there was no significant difference between those with diabetes and those without in frequency of occurrence. Only five specimens showed appreciable quantities of lipid in the arteries, three in the diabetic group and two among the non-diabetic group. (Table II.) In three of these specimens there was a marked lipophagic reaction, in the remaining two the vessels contained abundant oil red O-staining material without any cellular response. In general, the vessels in the choroid showed considerably more lipid than those in the retinal arteries. Interestingly, such lipophagic lesions gave a positive colloidal iron reaction, in contrast to the negative reaction for lipids and acid mucopolysaccharides in the proliferative lesions.

TABLE I
DISTRIBUTION OF CASES

Type of Patient	Up to 50 yr.		Over 50 yr.	
	No.	%	No.	%
Diabetic (Thirty-Four Patients)				
Male hypertensive	4	80	11	38
Male normotensive	1	20	4	14
Female hypertensive	0	0	13	45
Female normotensive	0	0	1	3
Total	5		29	
Non-Diabetic (Fifty Patients)				
Male hypertensive	4	36	14	36
Male normotensive	4	36	7	18
Female hypertensive	0	0	13	33
Female normotensive	3	27	5	13
Total	11		39	

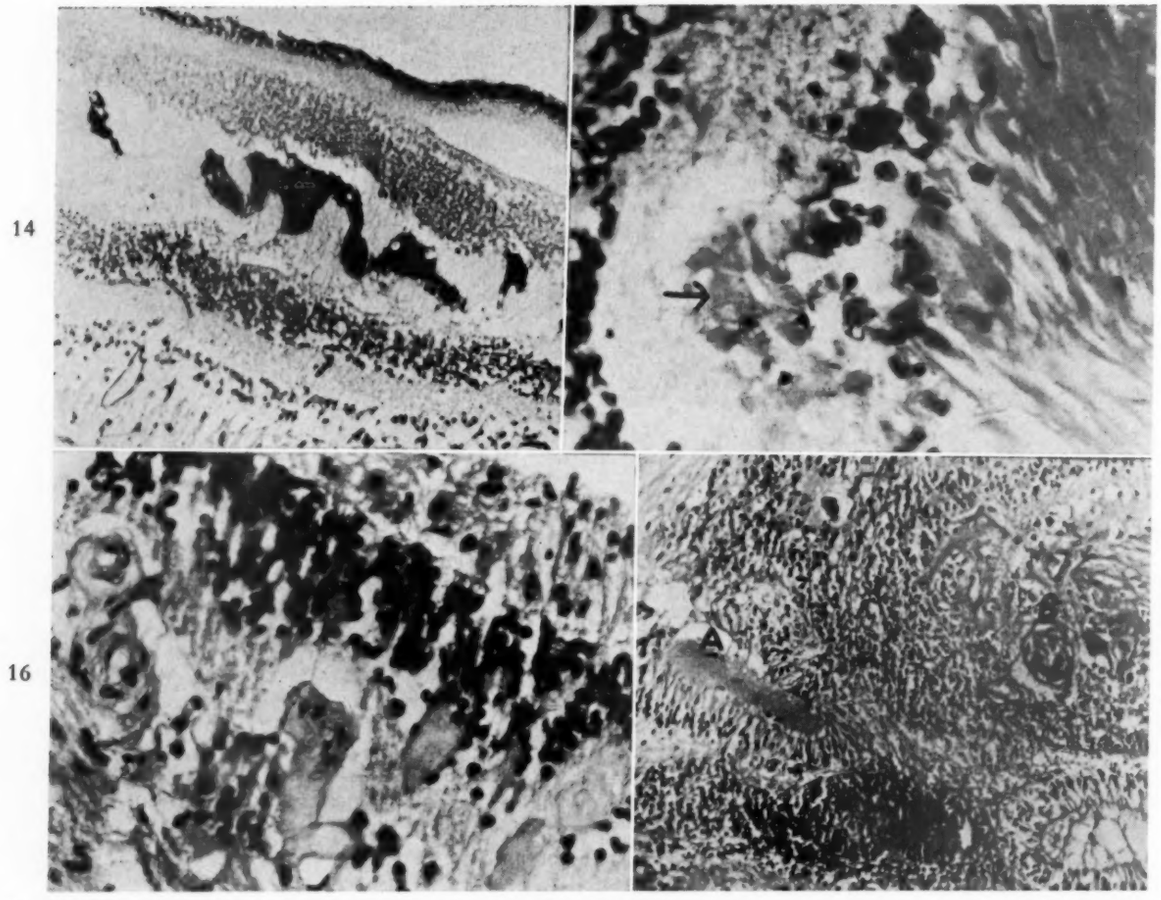

FIG. 14. Periodic acid-Schiff stain, original magnification × 200. Hard exudate is represented by darkly staining (PAS-positive) material between the two nuclear layers.

FIG. 15. Hematoxylin and eosin stain, original magnification × 500. Fibrinoid exudate is indicated by arrow. Note linear collection of plasma cells and lymphocytes along the right margin of the exudate.

FIG. 16. Hematoxylin and eosin stain, original magnification × 500. In the center is a multinucleated giant cell, and on both sides of it an infiltration of lymphocytes and plasma cells. Proliferative vascular reaction is seen along the left margin.

FIG. 17. Hematoxylin and eosin stain, original magnification × 150. This is from a patient with retinitis proliferans with retinal detachment. There is marked disarrangement of the architecture. *A*, an area of fibrinoid exudation, with cells arranged radially around it to resemble a "rheumatoid nodule." *B*, a proliferative vascular lesion.

Hemodynamic Lesions. We have used the term "hemodynamic" to designate those lesions generally considered to be arteriosclerotic in character but which lack a significant quantity of lipid, on the presumption that they are the result of the response of vascular tissue components to hemodynamic forces. As might be expected, in comparable age groups hemodynamic lesions occur with greater frequency among hypertensive subjects than among normotensive subjects, except in a few groups in which the number of cases is too small to be significant. In addition these lesions were, as a rule, more advanced among hypertensive subjects than among normotensive subjects. In the younger age group such hemodynamic lesions were found two to three times more frequently among diabetic patients than among non-diabetic subjects. In the older age group they were found 10 per cent more frequently in small vessels in non-diabetic subjects and 10 per cent more frequently in medium-sized vessels in diabetic patients. In general, hemodynamic lesions of small vessels occurred with slightly greater frequency among diabetic patients, and they were found most frequently in medium-sized vessels. These differences appear to be of about the same order of magnitude as the dif-

TABLE II

ANALYSIS OF INFLAMMATORY, THROMBOTIC AND ATHEROMATOUS LESIONS

Type of Patient	Inflammatory Lesion		Thrombotic Lesion		Atheromatous Lesion	
	Up to 50 yr.	Over 50 yr.	Up to 50 yr.	Over 50 yr.	Up to 50 yr.	Over 50 yr.
Diabetic						
Male hypertensive	0	4	1	1	2	4
Male normotensive	0	0	0	0	0	1
Female hypertensive	0	0	0	1	0	2
Female normotensive	0	0	0	0	0	1
Total	0/5	4/29	1/5	2/29	2/5	8/29
Grand total	4/34 = 12%		3/34 = 9%		10/34 = 29%	
Non-Diabetic						
Male hypertensive	0	0	0	1	1	1
Male normotensive	0	2	0	1	0	0
Female hypertensive	0	0	0	0	0	2
Female normotensive	1	1	0	0	0	0
Total	1/11	3/39	0/11	2/39	1/11	3/39
Grand total	4/50 = 8%		2/50 = 4%		4/50 = 8%	

ferences in the frequency of hypertension in the two groups.

Hemodynamic lesions of greater intensity than in the retina were seen in large arteries of other structures of the eye, but the data in Table II are limited to the retina.

Proliferative Lesions. Proliferative lesions were found almost nine times more frequently among diabetic patients than among non-diabetic subjects in the younger age group; in the older age group they occurred over 3.5 times more frequently among diabetic patients. While more common in hypertensive subjects in both groups, they were also found in normotensive subjects. Of the ten non-diabetic subjects showing such proliferative lesions there were two with a penetrating injury of the eye, one had pernicious anemia, one had glomerulonephritis, two had cirrhosis of the liver, and in the remaining four death was attributed to cardiovascular or cerebrovascular arteriosclerosis.

Non-Vascular Exudative Lesions. Two types of exudates have been described in connection with diabetes. The first, cotton wool exudates, consisting of accumulations of large lipid-laden macrophages, were seen only occasionally in both diabetic and non-diabetic groups. Hard waxy exudates were seen only in the diabetic group. Characteristically, they occurred in the outer plexiform layer, and were strongly PAS-positive (Fig. 14) and colloidal iron-negative. Proliferative vascular lesions were seen in every instance in which these hard exudates were found. In several specimens from diabetic patients, tissue degeneration resembling fibrinoid was found, usually in association with clusters of plasma cells. (Fig. 15.) The fibrinoid material was moderately PAS-positive, suggesting the possibility that this may be an early stage leading to formation of the hard waxy exudate. In occasional other eyes of diabetic patients, lymphocyte and plasma cell infiltration was found along with fibrinoid degeneration, sometimes in association with giant cell formation, and usually in a region of marked proliferative vascular reaction. (Fig. 16.) In some instances associated with retinal detachment there was a dissociation of cells of the nuclear layers,

TABLE III
ANALYSIS OF HEMODYNAMIC LESIONS

Type of Patient	Small (up to 30 μ)				Medium (over 30 μ)			
	Up to 50 yr.		Over 50 yr.		Up to 50 yr.		Over 50 yr.	
	No.	%	No.	%	No.	%	No.	%
Diabetic								
Male hypertensive	2/4	50	8/11	72	3/4	75	8/11	72
Male normotensive	0/1	0	2/4	50	0/1	0	2/4	50
Female hypertensive	0/0	...	6/13	46	0/0	...	9/13	69
Female normotensive	0/0	...	1/1	100	0/0	...	1/1	100
Total	2/5	40	17/29	59	3/5	60	20/29	69
Grand total	19/34 = 56%				23/34 = 68%			
Non-Diabetic								
Male hypertensive	2/4	50	13/14	93	1/4	25	7/14	50
Male normotensive	0/4	0	2/7	26	0/4	0	2/7	29
Female hypertensive	0/0	...	9/13	69	0/0	...	11/13	85
Female normotensive	1/3	33	3/5	60	1/3	33	3/5	60
Total	3/11	27	27/39	69	2/11	18	23/39	59
Grand total	30/50 = 60%				25/50 = 50%			

which were intermixed with lymphocytes and some plasma cells. In some foci these appeared to be radially distributed around a central area of fibrinoid degeneration, thus resembling a rheumatoid nodule. (Fig. 17.) However, it was difficult to determine how much of this configuration might be due to folding of the retina following its detachment and how much may represent a true reaction pattern.

COMMENTS

Several points of interest emerge from the foregoing observations: (1) Neither atheromatous nor hemodynamic lesions of the retinal arteries, both of which fall into the category of arteriosclerosis, were found in significantly higher frequency in diabetic patients than in non-diabetic subjects. This was particularly true of the hemodynamic lesions, when the factor of hypertension was properly assessed in making this comparison, a finding consistent with previous observations by Blumenthal et al. [*25–28*] on the purported intensification of the arteriosclerotic process in diabetic patients. This finding supports the observation of Ballantyne [*29*], Friedenwald [*14*] and Ashton [*3,30*] that arterio(or arteriolarsclerotic)sclerotic lesions do not necessarily precede the development of diabetic retinopathy. (2) While inflammatory and thrombotic lesions were found more frequently among diabetic patients, the former probably represented a secondary reaction to hemorrhage in many instances; thrombotic lesions were found in too few cases to warrant any conclusions. (3) Proliferative lesions of the retinal arteries occur, similar to those previously described in other anatomic sites in diabetic patients [*12,15,16*]. Furthermore, as in other sites, while these lesions were seen in non-diabetic subjects as well as in diabetic patients, they were

considerably more prevalent in the latter group. The difference in incidence in the retina between the two groups is of about the same order of magnitude as in other anatomic sites. (4) Histochemical studies indicate that these vascular lesions contain little or no demonstrable lipid or ceroid, and that the polysaccharide which is deposited in them and in the hard waxy exudates is predominantly of a non-sulfated type. As pointed out recently by Bertelsen [*31*], the latter might include neutral mucopolysaccharides containing hexosamine, neutral monosaccharides and glycoproteins which have bound hexosamine, as well as various neutral monosaccharides in a firm covalent bond to the protein. The term "neutral" implies merely the absence of hexuronic acids as constituents of the molecule. The histochemical reactions observed in this study are similar to those obtained previously in the nodular lesions of diabetic glomerulosclerosis [*12*]. (5) Certain secondary changes in the retina were found which suggest the possibility that an immunogenic reaction may be involved in the development of diabetic retinopathy. These consist of the appearance of a fibrinoid exudate which probably precedes the formation of waxy exudates, sometimes associated with lymphocyte and plasma cell infiltration or giant cell reaction.

TABLE IV
ANALYSIS OF PROLIFERATIVE LESIONS

Type of Patient	Up to 50 yr.		Over 50 yr.	
	No.	%	No.	%
Diabetic				
Male hypertensive	3/4	75	8/10	80
Male normotensive	1/1	100	2/4	50
Female hypertensive	0/0	...	10/13	77
Female normotensive	0/0	...	1/1	100
Total	4/5	80	21/28	75
Grand total	25/33 = 76%			
Non-Diabetic				
Male hypertensive	0/4	0	1/14	7
Male normotensive	0/4	0	4/7	57
Female hypertensive	0/0	...	4/13	31
Female normotensive	1/3	33	0/5	0
Total	1/11	9	9/39	23
Grand total	10/50 = 20%			

It may seem surprising that at this late date new pathologic observations can be made in vascular abnormalities of the eye, some of which were recognized as long ago as 1888 [*32*], and extensively studied since about 1934 [*33*]. However, most such studies have dealt with the gross pathologic characteristics of retinal aneurysms, as revealed by ophthalmoscopic examination and by the technic of examining unsectioned flat preparations of the retina under the microscope, first introduced by Ballantyne and Lowenstein [*34*]. Wagener et al. [*33*] described localized swellings of the veins without hemorrhage or exudate as early as 1934, an observation which was later confirmed by Ballantyne and Lowenstein [*21,29,34,35*]. Ballantyne [*36*] and also Ashton [*30,37*] showed that the earliest hemorrhages just outside the macular areas occurred in juxtaposition to the venous microaneurysms. These observations were confirmed by Friedenwald [*38*] through a study of the retinal lesions reconstructed in serial section, and by Friedenwald [*39*] and Day [*40*] utilizing the technic of unsectioned flat preparations of retina.

The presence of an endothelial vascular reaction was noted by Ballantyne and Lowenstein [*21*], but they and most other investigators have considered this phenomenon to be secondary to aneurysm formation. However, Gibson and Smith [*41*] have maintained that it is this proliferative process which causes the venous distention, and that the microaneurysms develop later. Some support for this contention may also be derived from the experimental observations of Becker and Post [*42*] that occlusion of the central vein leads to capillary changes and microaneurysm formation, but Friedenwald [*38*] pointed out that these are usually varicose or fusiform and rarely saccular.

More recent observations may serve to clarify the sequence and significance of the proliferative vascular lesion. The studies of Mendlowitz et al. [*43*], Megibow et al. [*44*] and of Ditzel and Sagild [*45*] indicate an impaired circulation in small vessels generally, in diabetes, even before nephropathy or retinopathy can be detected. In regard to the retinal circulation specifically, Hickam and Sieker [*46*] observed that the retinal arterioles of diabetic patients

may show little or no change on ophthalmoscopic examination and yet suffer marked functional impairment, as indicated by reduction in the constrictor response which is normally present. Furthermore, in relation to the kidney Bell [*47*] has maintained that there is a thickening of both the afferent and efferent arterioles in diabetic glomerulosclerosis, and that the presence of a lesion in the efferent arteriole serves to differentiate these changes from those seen in hypertension.

It appears likely that the proliferative vascular lesion herein described may account for such functional impairment of small arteries and arterioles. Since this functional impairment evidently may precede the detection of retinopathy or nephropathy, it follows that the proliferative lesion of the retinal circulation may precede the development of microaneurysms. Anoxia, venous stasis and capillary fragility have all been considered antecedents to the development of microaneurysms [*19,36,48,49*]. Ashton [*3,30*] has postulated that venous engorgement leads to tortuosity and twisting of the retinal veins, so that kinks or loops are formed. Exudation within the loop may then lead to fusion of the adherent surfaces, followed by dissolution of the fused walls and formation of the characteristic aneurysm, which protrudes only on the free surface of the retina. Ashton [*3,30*] recognizes the importance of an anoxic stimulus in initiating these events. Such anoxia could, of course, occur on the basis of functional vasospasm, but it would appear more likely that persistent anoxia would be necessary and this would presuppose an organic vascular lesion possibly of the type herein described.

Considerations of the etiology of the vascular disease of diabetes revolve around two findings: (1) certain histochemical characteristics of the retinal microaneurysms and nodular glomerular lesions, and (2) alterations in the concentrations of certain serum components in diabetes. As regards the first, the demonstration of lipid-containing substances in these two lesions, and the resemblance of the fundus in traumatic cases to that of diabetes [*50,51*] and of the glomeruli in choline-deficient rats [*52*], as well as in human subjects with fat embolism [*53*], have suggested the possibility of an embolic origin of the vascular abnormalities. Based on observations in only four cases of diabetes with retinopathy, Pope [*54*] recently offered the hypothesis that microaneurysms result from focal accumulations of fat in the capillary wall, with subsequent stretching of the latter so that a hernia is produced through the supporting reticulum network. Cook [*55*] has concluded that the resemblance of the retinal changes in fat embolism to diabetic retinopathy is only superficial, and the studies of Raphael and Lynch [*53*] have disclosed fewer lesions of the glomeruli in patients with fat embolism than in the characteristic nephropathy of diabetes. The strong PAS-reaction obtained in the retinal vascular and glomerular lesions has suggested a disordered metabolism of mucopolysaccharide, and this possibility is presently receiving some attention.

With regard to alterations in serum components, studies dealing with a variety of lipid substances have been largely inconclusive [*56–63*]. Gofman et al. [*62*] have shown an increased frequency of certain beta lipoproteins in diabetes, especially in diabetic subjects with vascular complications, and Keiding et al. [*57*], and Engelberg et al. [*59*] believe that beta lipoproteins are directly related to the development of diabetic retinopathy and nephropathy. However, a similar increase occurs in atherosclerosis in the absence of renal and retinal complications [*60,61,63*], in myxedema and nephrosis [*63*], and in malignant hypertension treated with pyrogens [*64*]. In regards to serum mucopolysaccharide levels, some workers have found a higher value for protein-bound carbohydrate in the serum of diabetic patients than in the serum of non-diabetic subjects [*65–71*]; others have even reported a progressive rise with the onset of retinal and renal complications [*66,69*]. However, Berkman has observed a similar rise in renal disease not associated with diabetes, and Gilliland et al. [*67,68*] in a variety of diseases not associated with diabetes. As Ashton [*3*] has pointed out, similar rises in lipoproteins and mucoproteins have been reported in the serum of non-diabetic subjects with a wide variety of diseases in which retinopathy (and neuropathy) does not occur. One is forced to the conclusion either that these alterations in serum do not constitute the only factor in the genesis of diabetic vascular disease, or that they merely reflect some other basic primary factor.

Becker and co-workers [*19,72,73*] consider an interaction of steroids of the adrenal cortex with vitamin B_{12} as possibly of fundamental etiologic importance. They cite work suggesting that adrenal cortical activity may be responsible for the abnormal metabolism of mucopolysac-

charides and lipoproteins. Thus far, however, attempts to produce retinopathy and nephropathy by the administration of adrenal cortical hormones and vitamin B_{12} have not produced convincing results.

Still another possibility as to fundamental etiology is suggested by the results of the present study. As we have recently indicated [*16*], the structural characteristics of the vascular lesion herein described resemble most closely those produced by immunogenic mechanisms. This is suggested further by certain of the accompanying reactions in the retina, such as fibrinoid degeneration and plasma cell infiltration. Support for this possibility also derives from the observation by Freedman et al. [*74*], utilizing fluorescence microscopy, that gamma globulin is localized in the glomerular nodules of diabetic nephropathy, and by the observation by Ditzel and Moinat [*75*] that there is an aggregation of erythrocytes in the microcirculation of the conjunctiva of diabetic patients which appears to be associated with an increase in serum $alpha_1$-, $alpha_2$- and beta globulins. Studies by other investigators suggest the possibility that an immunogenic mechanism involving an antibody against insulin may be responsible for the vascular complications. Dunner et al. [*76*] have demonstrated that insulin can produce retinal hemorrhages in dogs, although they did not establish that this occurs through leakage of aneurysms. Jahnke [*77*] has observed (and cites references to other instances) of insulin apparently provoking retinal changes in human diabetic subjects. He contends, further, that diabetic retinopathy has been increasing since the use of insulin as a therapeutic agent, and that this increase is not merely due to the prolongation of survival of diabetic patients. However, as Goto [*78*] has pointed out, these phenomena can also be explained as due to secondary stimulation of the adrenal cortex, an interpretation which may be taken as in support of Becker's concept. On the other hand, Bornstein and Hyde [*79*], in comparing two groups of patients with juvenile diabetes, have found a greater concentration of insulin antagonists in the serum of the group with vascular complications than in the group without such complicating disease.

Gilliland and Stanton [*68*] have found that an increase in serum mucoprotein concentration, such as is seen in diabetes, also occurs in rheumatoid arthritis, tuberculosis with associated amyloidosis, and rheumatic fever. Since these are conditions which are presently considered to be of possible immunogenic origin, it may be that the blood polysaccharide changes in diabetes also are a reflection of an immune process.

Recently [*80*] an exudative phase has been noted in the development of the lesion of diabetic glomerulosclerosis. It is characterized by the superimposition of a fibrinoid material upon the hyaline glomerular nodule. This lesion is seen not only with the glomerulosclerosis of diabetes but also with the contracted kidney, and with the nephrotic phase of glomerulonephritis. In diabetic patients it often occurs terminally and is associated with shock. The exudate appears to be histochemically similar to that seen with so-called collagen diseases, and is also similar to the fibrinoid material in the retina in the present studies, in which there was also an associated lymphocytic and plasma cell infiltration. These retinal changes were most pronounced in patients with retinitis proliferans, suggesting the possibility that this phase of diabetic retinopathy may represent the counterpart in the eye of the exudative phase of diabetic glomerulosclerosis in the kidney. Both are highly suggestive of an immunogenic response.

In considering the possibility that the vascular complications of diabetes may have an immunogenic origin, with insulin as the antigen, it is necessary to account for those patients who manifest such vascular phenomena but have never received insulin. LeCompte [*81*] has presented three cases of "insulitis" in patients with early juvenile diabetes and has reviewed the literature on this subject. This lesion has been found in patients who died during their initial diabetic coma. Lymphocytic infiltration into the islets of Langerhans constitutes the primary lesion, and Gordan [*82*] has suggested that this may represent an autoimmune phenomenon similar to that present in patients with lymphocytic thyroiditis. In recent years there has been a growing interest in the role of insulin antibodies in diabetes, particularly with respect to insulin allergy and resistance. The foregoing comments indicate a need for studying the possible role of such antibodies in the development of the vascular complications of diabetes.

The occurrence of microaneurysms is not limited to diabetes. Ashton [*3*] has cited a number of reports of their presence in the conjunctiva, and their occurrence there has been thought to be of significance in diabetes by McCulloch and Pashby [*83*] and Weinstein and

Forgacs [*84*], but not by others [*55*]. Retinal microaneurysms have been observed in a variety of diseases not associated with diabetes [*3,5, 85–87*]. These include Eale's disease, Coat's disease, pernicious and sickle cell anemia, adrenal cortical hyperplasia, retinal venous occlusion from a variety of causes, thrombotic glaucoma, chronic uveitis especially following perforating injury, retinoblastomas, choroidal melanoma, malignant hypertension, choroiditis, chorioretinitis, and even occasionally in otherwise normal retinas [*85*]. In one study Ashton [*85*] found microaneurysms in one-third of the eyes removed at surgery and in 29 per cent in a postmortem group. In general, aneurysms in the non-diabetic subjects tend to be few in number and to occur in the periphery of the retina, while in diabetic patients they are characteristically located near the optic disc. In the ten patients in the non-diabetic group of the present study the proliferative vascular lesion was found in two patients with penetrating eye injuries, two with cirrhosis of the liver, one with glomerulonephritis, one with pernicious anemia, and four with complications of hypertension and arteriosclerosis.

Similarly, glomerulosclerosis, even of the nodular type, may occur in non-diabetic subjects. One such case was described in the original report of Kimmelstiel and Wilson [*88*], and we have already referred to the study of Raphael and Lynch [*53*] who found similar lesions in non-diabetic subjects with fat embolism. It will be proper to apply the term "specific" only after an etiologic mechanism will have been defined which is itself specific for some aspect of the diabetic process.

SUMMARY

The frequency of inflammatory, thrombotic, atheromatous, hemodynamic and proliferative lesions of retinal vessels have been compared in thirty-four eyes of diabetic subjects and fifty eyes of nondiabetic control subjects. Inflammatory lesions occurred with a slightly greater frequency among the diabetic subjects, but this was attributed, in part, to reaction to hemorrhage which occurs more frequently in diabetic subjects. Thrombotic and atheromatous lesions were found only rarely in both groups. Hemodynamic lesions were found with slightly greater frequency among diabetic subjects, but the difference was of about the same order of magnitude as the difference in frequency of hypertension. Proliferative lesions were found over three and a half times more frequently in diabetic than in non-diabetic persons.

The etiology and pathogenesis of these proliferative lesions are discussed in relation to current concepts of the origin of the vascular complications of diabetes. It is pointed out that the proliferative vascular lesion most closely resembles similar lesions produced by immunogenic mechanisms. The possibility that an immunogenic mechanism may be responsible for the vascular complications of diabetes is discussed.

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Reviews

Recent Progress in Human Chromosome Analysis and Its Relation to the Sex Chromatin*

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RAPID developments in the use of atomic energy during and following World War II resulted in a considerable expansion of research in chromosome analysis, particularly in human beings. In 1956 Tjio and Levan [*130*] in the United States showed that the normal human chromosome number is forty-six rather than forty-eight. This startling demonstration, quickly confirmed by Ford and Hamerton [*37*] in England, swiftly evoked even greater interest and more intense activity in problems of human karyotype resolution. These more recent investigations have been greatly facilitated by the timely development of sorely needed refinements in technic. In contrast with older methods, the latter now provide adequate quantities of well preserved mitotic chromosomes for examination. Such preparations permit for the first time a reasonably detailed and accurate study of the morphologic characteristics of individual chromosomes and of their total number per nucleus.

A tangential impetus to chromosomal investigation arose from the momentous discovery by Barr and his associates [*5,8,93*] of the existence of a distinctive chromocenter, the sex chromatin, in intermitotic nuclei of females and its virtual absence from the nuclei of males. While the precise origin of the sex-chromatin body has not yet been definitely determined, there is abundant inferential evidence [*54,75,89,111*] indicating that under normal conditions it originates from fused heterochromatic portions of two X-chromosomes. However, recent observations suggest that in certain human sexual anomalies [*34,55,68*] and in some animals [*83,99*] it may be derived from a single (positively heteropycnotic) X-chromosome.

Extensive application of methods of nuclear sexing to clinical instances of hypogonadism and of various forms of intersexuality led to the unexpected discovery of certain discordances between nuclear sex and somatic appearance (chromatin-positive Klinefelter's syndrome and chromatin-negative Turner's syndrome, and the syndrome of testicular feminization). In addition, it was soon demonstrated that true hermaphrodites may be either chromatin-positive or chromatin-negative. Nevertheless, despite their great interest and provocativeness, these observations by themselves are limited in their diagnostic usefulness and shed very little, if any, light on the pathogenesis of the relevant sexual disturbances.

However, beginning in 1959 and continuing to date, there has been a relatively large number of reports in the literature of studies of human chromosomes in various clinical conditions. These investigations afford considerable promise that, with further technical improvements and with the accumulation of more data, much may be learned concerning the relationship of chromosomal abnormalities to certain congenital and hereditary disorders, with and without sexual involvement, as well as to neoplasia and to radiation exposure.

It is proposed to review here the newer work dealing with human chromosome analysis, to correlate it with nuclear sex-chromatin studies and to bring into perspective the implications for various branches of clinical medicine. Mindful of the fact that many physicians are unfamiliar with

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the esoteric and highly technical vocabulary of cytogenetics and heredity, a definition and explanation of terms follows. The appended list should be helpful in comprehending terminology as well as in recalling certain basic mechanisms and concepts. Detailed discussions of these and related topics are available elsewhere [*28,29,48, 116,119,123b,129,137*].

GLOSSARY

Acrocentric: Type of chromosome in which the centromere is located near one end. At metaphase it has the appearance of a "wishbone." Also called *subterminal.*

Alleles: A pair of genes, situated at a corresponding locus of a pair of homologous chromosomes, having to do with a specific characteristic in the offspring. A pair of alleles is generally indicated by the same letter, using a capital for the dominant, and a lower-case letter for the recessive. An individual possessing a pair of identical alleles, either the two dominants or the two recessives, is "pure" for the character controlled by the gene and is therefore *homozygous* for this gene. The union of contrasting genes, a dominant and its recessive allele, results in an *heterozygous* individual.

Allocycly: The specific differential staining reactivity of heterochromatin which permits its cytologic recognition and distinction from the remaining euchromatic portion of a chromosome. This phenomenon is apparently related to the cycle of the union of nucleic acid with the protein complex of the chromosome. Accordingly, its expression varies in the different phases of the mitotic cycle. In the X-chromosome it is most pronounced in prophase.

Anaphase: Phase of cell division characterized by the movement of chromosomes from the metaphase plate toward the opposite poles. In mitosis it is the longitudinally-doubled chromosomes (chromatids) which separate. In the first stage of meiosis whole chromosomes instead of chromatids segregate at anaphase. The second meiotic division is essentially mitotic, with separation of chromatids. (Fig. 1.) See *mitosis.*

Aneuploidy: State characterized by an irregular number of chromosomes, not an exact multiple of the basic number characteristic for the species. For example, the presence of 45, 47 or 48 chromosomes in man, caused by the loss or addition of one or more chromosomes. Such conditions are also known as *hypoploid,* and *hyperploid,* respectively. See *monosomic, trisomic.*

Autosome: A non-sex chromosome.

Centric fusion: A special type of reciprocal translocation [*137*] in which each of the involved chromosomes is broken very close to its centromere so that exchange of entire or virtually entire chromosome arms takes place ("whole-arm" translocation). When both of the involved chromosomes are acrocentric and breakage occurs in the long limb of one and in the short limb of the other, transposition of the arms produces a large submedian chromosome and a minute element containing a centromere and a very short region on either side. (Fig. 6.) Since each new chromosome possesses a centromere it is potentially viable. However, the minute fragment tends to be lost in subsequent generations. Thus centric fusion followed by loss of the minute element leads to a decrease in the basic chromosome number.

Centromere: A non-staining, apparently structureless constriction visible in chromosomes during prophase, metaphase and anaphase. It produces the so-called *primary constriction* of each chromosome and is the region at which the latter becomes attached to the spindle. Its position, which may be median (metacentric), submedian (submetacentric) or acrocentric (near one end), is constant for a given chromosome and determines its form. Accordingly it assists in the identification of individual chromosomes. Also termed *kenetochore.*

Chiasma: The point of contact between non-sister chromatids of paired homologous chromosomes seen during the prophase of the first meiotic division. It is the cytologic expression of completed genetic crossing over, the latter providing for the exchange of genic material between two chromosomes before they separate.

Chimera: An organism, usually a grafted plant, exhibiting a mixture of genetically different types lying adjacent to one another. This term has been applied to human subjects (twins) possessing more than one blood group, i.e., mixtures of A and O cells, who therefore exhibit *erythrocyte mosaicism* [*78*].

Chromatid: During nuclear division each chromosome presents a longitudinally-double form, each half of which is known as a chromatid. The components of a single chromosome are *sister chromatids* and are held together at the centromere. When these separate at mitotic anaphase they are called *daughter chromosomes.*

Chromatin: The substance in nuclei and chromosomes which stains intensely with basic dyes. It is composed of DNA combined with proteins. In the fixed intermitotic nucleus it usually takes the form of an irregular network of long coiled threads. Larger and denser chromatin particles are known as *chromocenters.* As a cell undergoes division the delicate coils of chromatin material are gradually transformed into individual chromosomes.

Chromatin-negative: Refers to nuclei which lack Barr's sex-chromatin mass. Characteristic of the normal human male.

Chromatin-positive: Refers to nuclei containing the distinctive sex-chromatin body of Barr. Present in the normal human female.

Chromocenter: Deeply staining clumps of chromatin material present in intermitotic nuclei. A chromocenter of characteristic size and shape lying in contact with the nuclear membrane is present in females of many species including man. It is known as the *sex-chromatin* body of Barr.

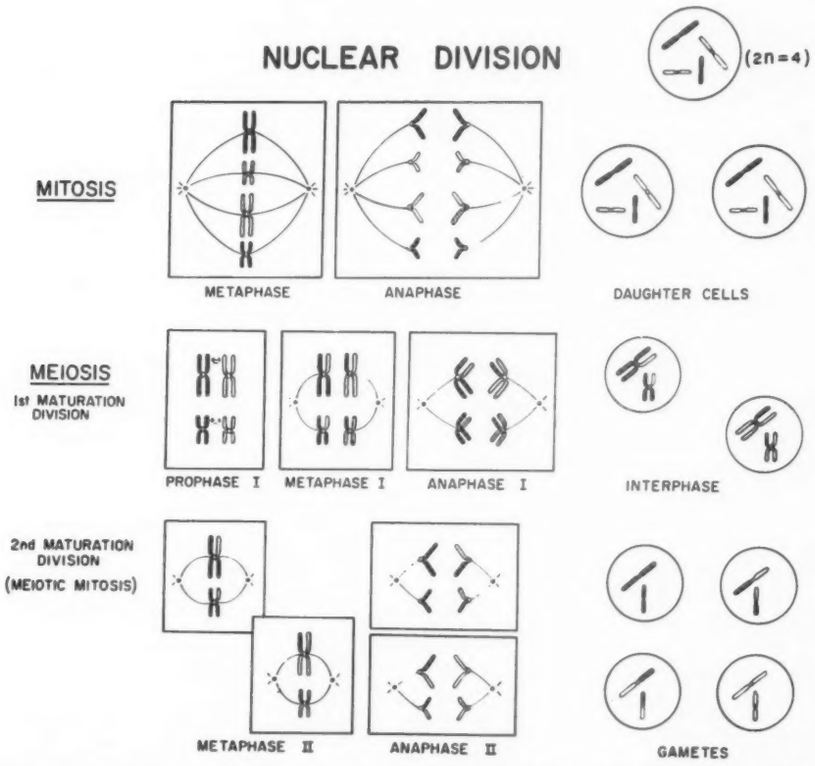


FIG. 1. Highly schematic representation of the distinguishing features of mitotic and meiotic nuclear division. The parent cell contains two pairs of chromosomes, the dark and light members of each pair being derived from the father and mother, respectively. In mitotic metaphase, each chromosome is reduplicated and arranged separately on the equatorial plate. During anaphase, the longitudinal halves of the chromosomes separate and pass to opposite poles producing daughter cells whose chromosomal constitution is identical with that of the parent cell. At metaphase of the first meiotic division, the reduplicated members of each homologous pair are together in synapsis on the equatorial plate. Exchange of chromosomal material (crossing over) has just taken place during prophase. During anaphase, whole reduplicated chromosomes of each pair diverge poleward to produce daughter cells containing half the diploid number. Metaphase of meiosis II is essentially mitotic in character, resulting in gametes containing the haploid number of chromosomes, but with varying compositions of parentally derived chromosome material.

Chromosome: One of a finite number of small bodies occurring in pairs into which the chromatin material of a cell nucleus resolves itself prior to cell division. Chromosomes are not visible as such except during cell division. *Homologous* chromosomes are the two members of one pair, one of maternal, one of paternal origin. At metaphase, the longest human chromosome measures approximately 8 to 10 μ and the smallest 1.2 to 1.5 μ. It is the unit structure bearing the carriers of hereditary traits, the genes. The morphologic characteristics of the individual chromosome and their total number is constant for all the somatic cells of a given species. Its major chemical components are DNA, RNA, histones and non-histone proteins.

Clone: A group of cells constituting the progeny by asexual reproduction from a single sexually produced cell. The clone is of importance in mosaicism, in which genotypically different ancestral cells give rise to genetically dissimilar stem-lines of daughter cells.

Crossing over: The exchange of chromatic material between synapsed homologous chromosomes during prophase of the first meiotic division. Occurring when the chromosomes are longitudinally double, the event involves non-sister chromatids and not whole chromosomes. It takes place between, not at, the loci of genes. The process is responsible for new recombinations of genes in the gametes which differ from the parental gene combinations in the original germ cells.

Deletion: A chromosomal aberration characterized by detachment and loss of a portion of a chromosome by breakage. In this manner one or more genes may be removed from the organism. The deleted portion fails to survive if it lacks a centromere.

Diploid: The number of chromosomes present in all somatic and primitive germ cells of a species. It is referred to as *2n* where *n* signifies the haploid (half) number which is found in gametes.

Dominant: Refers to a gene which produces its effect despite the presence of an opposite or contrasting gene.

Duplication: A type of chromosomal aberration characterized by the presence of an extra segment of

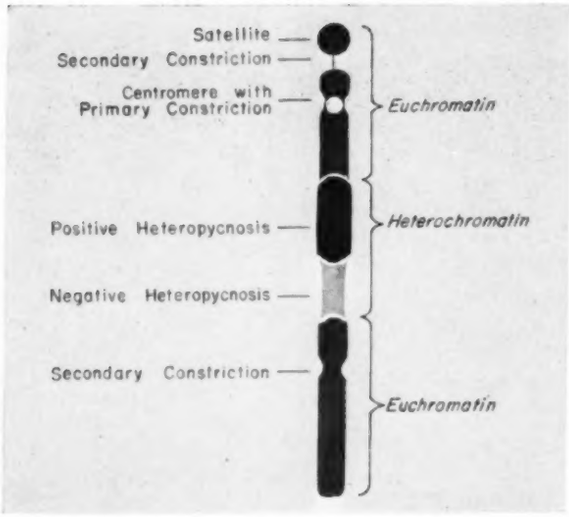


FIG. 2. Schematic representation of certain morphologic and differential staining characteristics found in some but not all chromosomes. (A centromere and its primary constriction are present in *all* human normal chromosomes.) See Glossary for explanation of terms.

chromosome which may exist as a separate fragment or may be attached to one of the members of the ordinary chromosomal complement. To be distinguished from *aneuploidy* and *polyploidy* in which variations in somatic chromosome number are irregular or exact multiples, respectively, of the basic chromosomal number.

Equational division: The second meiotic division, essentially mitotic in type, characterized by the separation of sister chromatids. The latter are genetically identical since they are longitudinally-split reduplications of individual chromosomes. (Fig. 1.)

Equatorial plate: A circular disc-like area situated at right angles to the spindle fibers of the dividing cell. On it are arranged the chromosomes preparatory to their separation (in meiosis I) or to the separation of their chromatids (potential daughter chromosomes) in mitosis. Also known as the *metaphase plate*.

Euchromatin: The chromatin material composing a chromosomal segment which exhibits no variation in staining intensity (isopycnosis) or in condensation at any time during the mitotic cycle. (Fig. 2.) Most if not all genes directly responsible for hereditary transmission are believed to be situated in this type of chromatin. Contrasted with *heterochromatin*.

Euploidy: State in which there is a balanced set of chromosomes. Includes variations in the somatic chromosomal number characterized by the presence of exact multiples of the basic number of that species. Accordingly, these may be *haploid* (*monoploid*), *diploid*, *triploid*, *tetraploid* and higher multiple numbers. Complements higher than diploids are called *polyploids*.

Expressivity: The regularity in different individuals with which a gene produces a trait. Expressivity of a gene may therefore be variable (as for allergy) or constant (as for blood groups). Differs from *penetrance* which relates to the statistical regularity with which a gene produces its effect.

Gamete: In bisexual organisms, the mature germ cell of either sex (spermatozoa or ova) containing the haploid (n) number of chromosomes.

Genes: The basic ultramicroscopic, intramolecular units of heredity arranged linearly at definite points (loci) in a chromosome. They occur in pairs (*allelic* genes) situated at corresponding loci of a pair of homologous chromosomes and are capable of producing externally visible body traits composing the phenotype. Apparently, genes function by controlling enzymatic action.

Genotype: The genetic constitution of an organism regardless of the external appearance. Thus, a genetic female may have the physical appearance (*phenotype*) of a male, and vice versa. Because of the Mendelian phenomena of segregation and recombination, together with exchanges of genes occurring during meiosis, it is probable that no two individuals (barring identical twins), in the course of mankind's existence, have ever had the same genotype [*136*].

Gynandromorphism: The condition of sexual mosaicism in an organism of a bisexual species. Such an individual possesses male and female tissues, with corresponding chromosomes of both sexes, in different parts of the body other than the gonads. This abnormality is usually due to chromosomal aberrations (such as the simple loss of one of the X-chromosomes) occurring during embryologic development. However, in some species, such as the silk-worm, it may be gene-determined.

Haploid: The half number (n) of the basic chromosomal complement (diploid or $2n$) of a species. The haploid number consists of one of each pair of chromosomes and is the normal condition in mature germ cells.

Hemizygous: Normal diploid cells, with their pairs of homologous chromosomes and genes, are either *homozygous* or *heterozygous* for each pair of genes, depending on whether the alleles of a pair are identical or contrasting. The *hemizygous* condition refers to the presence of an unpaired gene or chromosome. Haploid gametes, which normally contain a single set of chromosomes, are therefore hemizygous for the genes carried in these chromosomes. Also, the normal diploid heterogametic zygote, containing the partially or totally nonhomologous X- and Y-chromosomes, is hemizygous for the genes represented in the nonhomologous segments of the dissimilar sex chromosomes. Hemizygosity, as an abnormality, exists in *monosomic* cells in which only one member of a pair of homologous chromosomes is present. Such cells are accordingly hemizygous for the genes located in this particular chromosome.

Hermaphroditism: The intersexual condition in which male and female genital structures exist in an individual of a bisexual species. An intersex is a *true hermaphrodite* when gonadal tissue of both sexes is present,

and a *pseudohermaphrodite* (male or female) when the gonads contain either testicular or ovarian tissue respectively, but not both. In either case, varying combinations of male and female development are found in the genital tract.

Heterochromatin: The chromatin material occupying a chromosomal segment which exhibits variations in condensation and in staining intensity (heteropycnosis). This is in contrast to the remaining euchromatic portion of the same chromosome which shows no such variation. (Fig. 2.) The heterochromatic regions of chromosomes may appear thick and intensely stained (*positive heteropycnosis*) or thin and weakly stained (*negative heteropycnosis*). Heterochromatin is generally believed to be lacking in genes directly concerned with hereditary transmission although it probably exerts a modifying effect on near-by genes in the euchromatic zone. At metaphase it is usually indistinguishable from euchromatin. Heterochromatin is of particular interest in the X-chromosome in which (in man) it may occupy the entire length of the short arm.

Heterogametic: Refers to the XY or XO sex which produces two kinds of gametes (X, and Y or O) in equal numbers, whereas the XX sex is said to be *homogametic* since it produces only one kind of gamete (X). In most bisexual species the male sex is heterogametic so that there are two kinds of sperms (X- and Y-containing) but only one kind of egg (X-containing). In certain insects, birds and fishes the reverse is true, the females being heterogametic and the males homogametic.

Heteropycnosis: The phenomenon exhibited by the heterochromatic region of a chromosome characterized by variations in the degree of condensation and in the intensity of staining. (Fig. 2.) When the heterochromatic segment appears thicker and stains more intensely than the rest of the chromosome it is said to show *positive heteropycnosis*. This is particularly evident at prophase but these regions may also remain condensed during interphase where they are recognizable as chromocenters. Positive heteropycnosis is usually developed to a greater degree in sex chromosomes than in autosomes. When the heterochromatic zone appears thinner and more faintly stained than the remainder of the chromosome, it exhibits *negative heteropycnosis*. The heterochromatic region of a chromosome may be positively heteropycnotic at one stage of the mitotic cycle and negatively heteropycnotic at another stage of the same cycle.

Heterozygous: Refers to a zygote having a pair of dissimilar or contrasting alleles controlling a certain trait or character. When the two members of a pair of genes are alike, the organism is *homozygous* for that trait.

Holandric: Refers to genes situated in that portion of the Y-chromosome which does not pair with a homologous segment of the X-chromosome. The concept of holandric genes is not universally accepted [*123*]. However, if they do exist, such genes would be transmitted from an affected father to all his sons and the trait caused by such a gene will not occur in women. They are therefore completely *sex-linked*, since no crossing over occurs. Genes for certain rare skin conditions, hypertrichosis of the ears and webbed toes are claimed to be examples of holandric genes [*119*].

Homogametic: See *heterogametic*.

Homozygous: See *heterozygous*.

Hybrid: The progeny of two parents who differ genetically in any way. Also termed a *cross*.

Hyperploid: The aneuploid condition in which there is more than the normal diploid number of chromosomes, as exemplified by the *2n* plus 1 or *2n* plus 2 state in man with 47 or 48 chromosomes. See *aneuploidy*, *trisomic*.

Hypoploid: The aneuploid condition in which there is less than the normal diploid number of chromosomes, for example, the *2n* minus 1 state in man with 45 chromosomes. See *aneuploidy*, *monosomic*.

Idiogram: A diagrammatic representation of the chromosome constitution (karyotype) of the cells of an individual. Customarily, the chromosomes are systematically arranged according to size and centromere-position. (Fig. 5.)

Intermitotic: The state of a resting nucleus, i.e., when the cell is not undergoing division. Chromosomes, as such, are not visible during this phase. Actually, the nucleus is not "resting" since biochemical activity is at its height at this time. Also termed *interphase*.

Interphase: See *intermitotic*.

Intersex: See *hermaphroditism*.

Inversion: A structural chromosomal aberration characterized by the rearrangement (reversal) of a portion of the gene sequence. It occurs as a result of the breakage of a chromosome at two points followed by a reversal of the replaced fragment with respect to the rest of the chromosome.

Isochromosome: An abnormal chromosome arising through a misdivision of the centromere [*24b*]. Normally, during separation of the chromatids at anaphase the centromere divides in the longitudinal axis of the chromosome so that the daughter chromosomes are identical with one another. However, if the centromere misdivides, i.e., in a transverse rather than in a longitudinal plane, each of the two separated centromere fragments remains attached to the two chromatids of one arm. Thus, the two new chromosomes (isochromosomes) differ from one another. Each is composed of two arms of equal length and the arms are genetically homologous with each other. It is possible that this type of chromosome aberration, known to occur in maize, may also occur in man. In this event it might help to explain certain unusual or peculiar karyotypes in which there is an extra median chromosome.

Karyotype: A group of characteristics (number, size and form) used in identifying a particular chromosome constitution. For purposes of demonstration, a

karyotype is a systematized array of the metaphase chromosomes of a single cell typifying the chromosomes of an individual. It is customary to arrange the chromosomes in descending order of size and according to the position of their centromeres. (Fig. 3 and 4.)

Kinetochore: See *centromere.*

Linkage: Technically, a term applied to two different genetic phenomena: linkage between a gene and a chromosome, and linkage between genes. Accordingly, it may refer to the location (linkage) of a single specific gene in sex chromosomes (see *sex-linked, holandric*) or in autosomes. (Most hereditary disorders in man are determined by autosomal genes.) On the other hand, the term also refers to the association of two or more genes (such as those for body color or wing size in Drosophila) which tend to remain together during hereditary transmissions because they are located on the same chromosome. However, linked genes are capable of separation by means of crossing over. In the latter event, such genes show *incomplete* linkage to each other. An example of linked pairs of genes in human autosomes is that connecting the genes for the M-N blood types with the one for sickle cell anemia.

Median: Refers to the location of the centromere of a chromosome midway between its two extremities. The arms of the chromosome are therefore of equal length. Also termed *metacentric.*

Meiosis: A specialized type of cell division characterized by a mechanism which provides for a reduction in the number of chromosomes from the somatic or diploid number to the haploid number in the sperm and eggs, fertilization then restoring the somatic number in the zygote. Meiosis consists of two different types of division. The first, designated as *meiosis* I, is known as the reductional division because separation of members of homologous pairs of chromosomes results in a reduction in the chromosome number. The second meiotic division, *meiosis* II, is the *equational* division which is essentially mitotic in type, involving the separation of equally constituted sister chromatids. (Fig. 1.)

Metacentric: See *median.*

Metaphase: Phase of cell division characterized by complete disappearance of the nuclear membrane and by formation of the spindle. At this time the chromosomes are maximally contracted and are arranged on the equatorial plate preparatory to separation. In mitosis, the metaphase chromosome has a single centromere which becomes functionally double only at anaphase when the chromatids begin to move toward opposite poles. In the first meiotic division, which consists essentially of separation of paired homologous chromosomes, each bivalent has two centromeres. In the second meiotic division, metaphase is similar to that of ordinary mitosis. (Fig. 1.) See *mitosis.*

Mitosis: The process of cell division, in which the chromosomes and their genes participate, resulting in daughter cells which are qualitative and quantitative hereditary counterparts of the parent cell. It is divided into five stages: *Interphase* (resting), a relatively prolonged *prophase*, a brief *metaphase* and *anaphase*, and a *telophase* of intermediate duration. In man, as judged by observations in HeLa cells, a complete cycle consumes approximately twenty-four hours, the interphase lasting about twenty hours, prophase three hours, and the remaining phases a matter of minutes.

Monosomic: An aneuploid individual in whom there is loss of one of the two members of a pair of homologous chromosomes. When it occurs spontaneously, this condition results from meiotic *non-disjunction*, i.e., failure of the members of a pair to separate at anaphase, so that one resulting gamete contains both members of the pair (*n* plus 1) while the other lacks either member (*n* minus 1). Upon union with a normal gamete at fertilization, the offspring are therefore either *2n* plus 1 (*trisomic*) or *2n* minus 1 (*monosomic*), respectively, for this particular chromosome. These conditions are also referred to as *hyperploid* and *hypoploid*, respectively. *See aneuploidy.*

Mosaic: The presence of genetically dissimilar cells in adjacent tissues of an organism. Equivalent to *chimera.*

Mutation: A change in a gene. The mutant gene is capable of transmission and thus of producing hereditary variations when it is present in germ cells. Mutation may also take place in somatic cells, in which case the mutant gene cannot be sexually transmitted. Mutant genes are usually recessive to the wild type. Mutations may occur spontaneously or they can be induced by radiation by ionizing and other electromagnetic waves (x-ray, radium, ultraviolet, supersonic, ultra-short radio waves) and by chemical mutagens such as nitrogen mustard.

Non-disjunction: A type of abnormal chromosomal behavior during nuclear division. (Fig. 7.) It may occur in mitosis or in meiosis and is characterized by the failure during anaphase of the longitudinally-doubled chromosomes or of the members of a pair of homologous chromosomes to separate. As a result, one daughter cell receives both chromosomes while the other acquires none. Meiotic non-disjunction may be primary or secondary. *Primary* non-disjunction occurs in one or both parents, with the production of gametes containing an abnormal chromosomal constitution. *Secondary* non-disjunction refers to its occurrence during meiosis in an individual who is abnormal *ab initio*, being a product of the union of gametes, one of which was non-disjunctional.

Penetrance: Refers to the regularity with which a gene produces its effect. Penetrance is said to be *complete* if a dominant gene produces an effect in each person carrying it or if a recessive gene causes its effect in each individual homozygous for it. It is said to be *reduced* if some but not all persons exhibit the trait even though the gene is present in the required

heterozygous or homozygous stage. Compare with *expressivity*.

Phenocopy: A reproduction or copy of a gene-produced phenotype which is visibly indistinguishable from it. It occurs in the offspring of pure normal individuals as a non-hereditary modification produced by certain experimental conditions [*48*].

Phenotype: The apparent or externally visible type with characteristics which may be independent of the hereditary type (*genotype*).

Polyploidy: The presence of exact multiples of the basic haploid number of chromosomes higher than that found in the diploid state. This increase in chromosome number is due either to non-disjunction or to reduplication of the number of chromosomes in a somatic tissue with suppression of cytoplasmic division. In either case there results the addition of one or more complete chromosomal complements so that an individual may possess three (*triploidy*) or four (*tetraploidy*) or more of each kind of chromosome. See *euploidy*.

Polysomic: An aneuploid individual in whom one or more chromosomes are reduplicated and are found represented three or more times. The abnormal number of chromosomes results from meiotic non-disjunction which, when only one extra chromosome is involved, produces a gamete containing *n* plus 1 chromosomes. When this abnormal gamete unites at fertilization with a normal germ cell (containing the haploid or *n* number), the resulting zygote has a *2n* plus 1 chromosomal constitution. Such a polysomic organism is also designated trisomic for the non-disjunctional chromosome. *See aneuploidy*.

Proband: The index patient, an individual with an abnormality whose relatives are studied in an effort to ascertain the hereditary or genetic aspects of the trait. Also termed *propositus*.

Prophase: The first phase of cell division characterized by the transformation of the irregular network of chromatin material of the interphase nucleus into spirally coiled threads which gradually become doubled, shortened and thickened to form individual chromosomes. This phase terminates with the disappearance of the nuclear membrane, at which point metaphase starts. See *mitosis*.

Propositus: See *proband*.

Pseudohermaphroditism: Sexual anomaly characterized by the presence of the gonads of only one sex, but associated with elements of both sexes in the genital tract. Those individuals whose gonads are testes are male pseudohermaphrodites while female pseudohermaphrodites have ovaries. See *hermaphroditism*.

Recessive: Refers to a gene which fails to produce its effect in the presence of the opposite or contrasting gene. A recessive gene produces its effect only when both members of a pair are recessive, i.e., when the individual is *homozygous* for this particular gene, having received it from both parents.

Recombination: The union of two members of a pair of genes for a given character. This occurs at fertilization, each gamete contributing its single member of a pair of genes. See *segregation*.

Reductional division: The first meiotic division, characterized by the mere separation of members of homologous pairs of chromosomes, resulting in a reduction in the chromosome number from the somatic or diploid number to the haploid number. (Fig. 1.)

Satellite: A small rounded body attached to the end of certain chromosomes by a delicate thread of chromatin. (Fig. 2.) These terminal bodies are produced by a *secondary constriction* in the distal portion of an arm of certain chromosomes. (The *primary constriction* is caused by the centromere.) Satellites and their filaments are constant in their form and size for each particular chromosome. Therefore, they provide landmarks for the identification of these chromosomes. In man (Fig. 5) satellited chromosomes are the acrocentrics number 13, 14 and 21 (Denver system of nomenclature). Recent evidence suggests that *all* acrocentric chromosomes are satellited.

Segregation: The process in sexual organisms, constituting the Mendelian first law of inheritance, by which only one of the two genes of a pair gets into each mature germ cell. Then, when gametes unite at fertilization, two genes for each trait or character are brought together in the new individual (*recombination*). Segregation takes place during the reductional stage of meiosis at which time homologous chromosomes separate, each carrying with it a single member of a pair of genes. Each pair of genes segregates independently of other pairs so that varying combinations (in gametes) and recombinations (in zygotes) are produced (Mendel's second law of inheritance, that of independent assortment).

Sex chromosomes: The chromosomes, usually two in number although in certain organisms there is only one, which are concerned with the determination of sex of the offspring. In this respect they differ from all other chromosomes which are known as *autosomes*. There are two distinct sex chromosomes, the large X-chromosome and the small Y-chromosome. In many animals, including man, the nuclei of females contain the XX pair while the XY condition prevails in males. In some animals the female has two X-chromosomes but the male has one X- and no Y-chromosome. This is referred to as the XO type of male. In the case of the XY male, each sex chromosome is composed of two regions, the homologous portion known as the *pairing segment* and the non-homologous, non-pairing region designated as the *differential* segment.

In certain insects, birds and fishes the male has the equivalent of two X-chromosomes while the XY combination is found in the female. In such cases the sex chromosomes of the homogametic male are designated as ZZ and those of the heterogametic female as ZW. The W chromosome is absent in some species, in which case the heterogametic female is ZO (com-

parable to the XO heterogametic male of some other species).

Sex-influenced: Refers to genes which are dominant in one sex and recessive in the other, such as those for baldness and for one form of white forelock.

Sex-limited: Refers to genes, the penetrance of which is completely reduced in one sex, i.e., they are capable of producing traits in one sex but not in the other. The expression of these genes, at least in mammals and birds, apparently is dependent on the presence or absence of sex hormones [*119*]. Examples of sex-limited characters include the coloring of certain butterflies and the plumage of birds.

Sex-linked: Refers to genes located in a sex chromosome, X or Y. The best known sex-linked genes in man are those for red-green color blindness and hemophilia. These mutant genes are situated in the X chromosomes and since they are recessive they express themselves predominantly in males. Genes located in the Y chromosome are known as *holandric* genes. See *holandric*.

Spindle: A fusiform bundle of delicate filaments appearing at the beginning of the metaphase stage of cell division. It extends from the centriole of one pole of the dividing cell to that of the other. Some of the spindle fibers are continuous from pole to pole while others are attached to the chromosomes at their respective centromeres. The latter fibers may be responsible for bringing the chromosomes onto the metaphase (equatorial) plate.

Submedian: Refers to the location of the centromere of a chromosome at a point elsewhere than at the center or near the end.

Supernumerary chromosome: One or more extra chromosomes found inconstantly in wild populations of certain species of animals. They are not homologous to members of the regular set of chromosomes and they apparently exert little if any noticeable phenotypic effect. Certain supernumerary chromosomes frequently exhibit non-disjunction leading to their elimination from germinal as well as from somatic cells.

Synapsis: The period in meiosis in which the two members of every pair of homologous chromosomes are in very precise and intimate apposition. This occurs during prophase of the first meiotic division and implements the mechanism for the segregation and exchange of the maternal and paternal derivatives of every chromosomal pair. (Fig. 1.) The extent of synapsis between the heteromorphic *sex chromosomes*, X and Y, is less well understood and varies in different species. In man there is little if any normal pairing between the X and Y chromosomes, suggesting the absence of true homology between them [*21,112*].

Syngamy: In sexual reproduction, the union of germ cells (gametes) at fertilization to produce a new individual, a zygote.

Telophase: The final phase of cell division during which the chromosomes regroup into a nuclear structure and are gradually transformed into long thread-like fibers enclosed by a nuclear membrane.

Tetraploid: An organism containing four complete sets of each kind of chromosome characteristic of the species. Thus, the tetraploid number in man is 92. See *euploidy, polyploidy*.

Translocation: A type of aberration characterized by fragmentation of a chromosome and transfer of the broken-off portion to another chromosome, often of a different pair. This phenomenon is usually *reciprocal*, involving breakage of two chromosomes with mutual interchange of the fragments. (Fig. 6.) Reciprocal translocation, with the exchange of parts by two chromosomes belonging to different pairs, differs from *crossing over* in that the latter involves the exchange of *homologous* segments of *homologous* chromosomes. (Fig. 1.) The two new (translocated) chromosomes will survive and function if each possesses a single centromere. If a translocation-produced chromosome lacks a centromere, or if it should have two centromeres, it will not survive because proper separation during anaphase of a subsequent cell division will not occur.

Triploid: An organism containing three complete sets of each kind of chromosome characteristic of the species. Therefore, the triploid number in man is 69. See *euploidy, polyploidy*.

Trisomic: An aneuploid (hyperploid) individual in whom a chromosome is present in triplicate rather than in the normal double or disomic condition. The abnormal number of chromosomes results from meiotic *non-disjunction* (failure of the members of a pair of homologous chromosomes in the first meiotic division, or of the longitudinally-doubled chromosomes in the second division, to separate at anaphase) as a result of which one gamete contains both members of the pair (n plus 1). When this abnormal gamete unites at fertilization with a normal germ cell (containing the haploid or n number), the resulting zygote has a $2n$ plus 1 chromosomal constitution and the individual is therefore trisomic for the non-disjunctional chromosome. See *aneuploidy*.

Zygote: In bisexual organisms, the cell resulting from the union at fertilization of two germ cells. The formation of the zygote restores the normal diploid condition by combining the haploid number of chromosomes present in each of the gametes.

RECENT TECHNICAL ADVANCES

Until very recently the study of human chromosomes had been hampered by the fact that in standard histologic preparations these small structures tend to be clumped together and to overlie one another. Under such conditions it is often difficult to establish an accurate count and quite impossible to identify the individual chromosomes according to their morphologic characteristics. Since chromosomes can be recognized as such only during nuclear division

at or near the metaphase, the relative scarcity of adequate numbers of nuclei undergoing active mitosis in normal man constitutes an additional impediment. For this reason studies of human chromosomes had been almost limited to testicular material, in which spermatogenesis provides numerous spermatogonia and spermatocytes undergoing active cell division.

The introduction of combined new technics has made it feasible to analyze the chromosomal constitution of somatic as well as germinal cells, and with much greater accuracy. These procedures involve (1) the use of short-term cell cultures which provide growing and actively dividing whole cells with no distortion, loss or addition of cellular material by sectioning; (2) the use of hypotonic solutions to produce swelling of the cells with dispersion and contraction of the chromosomes; (3) treatment of the cell culture with colchicine which arrests mitosis at the metaphase and so enhances the number of cells suitable for examination; and (4) careful squashing to separate the individual cells or air-drying to spread the chromosomes. Camera lucida drawings and photographic prints at a magnification of 3,000 diameters make it possible to sort and match in pairs the individual chromosomes.

Cell culture technics for human chromosome analysis are now available for bone marrow [*35,38,113,114*], skin [*56,109*] and circulating leukocytes from peripheral blood [*94*]. The culture periods for these different tissue derivatives are several hours, approximately two weeks and three days, respectively. Unusual opportunities are now afforded for the investigation of a great variety of hereditary, congenital and neoplastic diseases by the collaborative efforts of clinicians and trained cytologists. It still remains to be seen, however, which type of tissue cell (or combination of tissues) affords the most accurate and complete information.

THE KARYOTYPE OF NORMAL MAN

Since 1956 there has been almost universal agreement that the normal diploid number of chromosomes, at least in European and American white persons, is forty-six. The matter of ethnic variations, not yet resolved, was introduced in 1957 by Kodani [*81,82*] who described small supernumerary chromosomes in non-cultured fixed testicular preparations (spermatogonial and first meiotic metaphases) of six of fifteen Japanese men and in one of eight white men in Iowa. Among the Japanese, nine had forty-six, one had forty-seven and five had forty-eight chromosomes while seven of the white men had forty-six and one had forty-eight chromosomes. A set of twenty-three pairs was present in all the testes, some having one and others two extra chromosomes. Since no homology existed between these additional chromosomes and any of the basic set they are to be regarded as supernumeraries rather than as trisomics or tetrasomics [*22*]. Although Makino and Sasaki [*92*] could not confirm the presence of extra chromosomes in tissue cultures of thirty-nine Japanese aborted embryos or in non-cultured squash preparations of six fresh fetuses, Kodani's observations on testes cannot be gainsaid. A possible explanation for the discordance between the results of Kodani and those of all other investigators may be, as suggested by Stern [*123a*], that supernumerary chromosomes may exist only in germinal tissue with subsequent elimination of these structures from somatic cells. However, while this phenomenon exists in certain lower animals, it is not known to occur in mammals. Moreover, somatic chromosome elimination could not be demonstrated by Chu and Giles [*21*] who performed chromosome determinations in cultures of germinal and somatic cells of the same individual and found identical chromosome numbers in each.

Human chromosomes at metaphase are recognizable as longitudinally-doubled rod-like structures, the halves of which are joined only at the centromere. (Fig. 3 and 4.) Each chromosome has a mate somewhere in the nucleus which can be matched with it, the two constituting a homologous pair. Homologous autosomes from cells of the same or of different individuals do not differ significantly either in relative length or in centromere position [*21*]. The individual members of a pair are identical in appearance except for the sex chromosomes of the male. In this instance, the twenty-two pairs of autosomes can be identified, the one large and one small remaining chromosomes being the sex chromosomes, X and Y, respectively. In the female, of course, the two X chromosomes constitute a matched homologous pair.

The human karyotype is obtained by sorting and matching the individual mitotic chromosomes of several single cells as visualized in drawings or in photographic enlargements. The pairs of chromosomes are then systematically arrayed according to their lengths and centro-

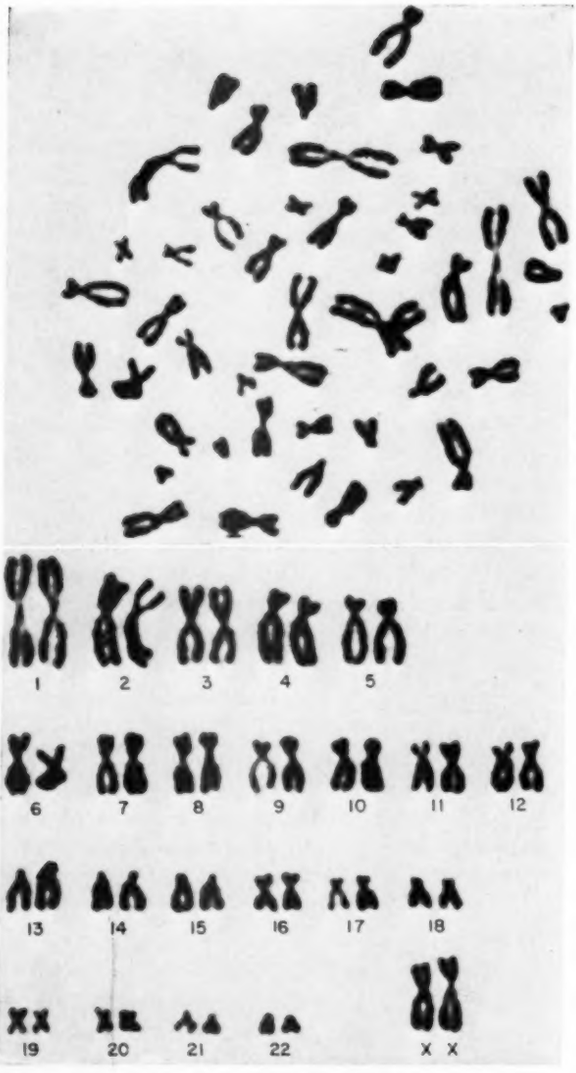


FIG. 3. *Top*, appearance of the chromosomes of a dividing human cell at metaphase. Each chromosome is longitudinally doubled, the chromatids (potential daughter chromosomes) being held together at their respective centromeres. *Bottom*, karyotype of a normal human female showing twenty-two pairs of autosomes and an XX sex chromosome constitution. This was constructed from the preparation above by sorting and matching homologous pairs of chromosomes. From: BARR, M. L. and CARR, D. H. *Canad. M. A. J.*, 83: 979, 1960 [*9*].

mere positions, the entire "true to life" chromosomal complement constituting the karyotype. The term *idiogram* refers to the diagrammatic representation of a karyotype.

Satisfactory karyotypes and idiograms have been published by several independent groups of investigators [*15,21,38,86,91,131*]. While these are in good general agreement, the use of several different systems of nomenclature resulted in confusion in the literature. Accordingly, a con-

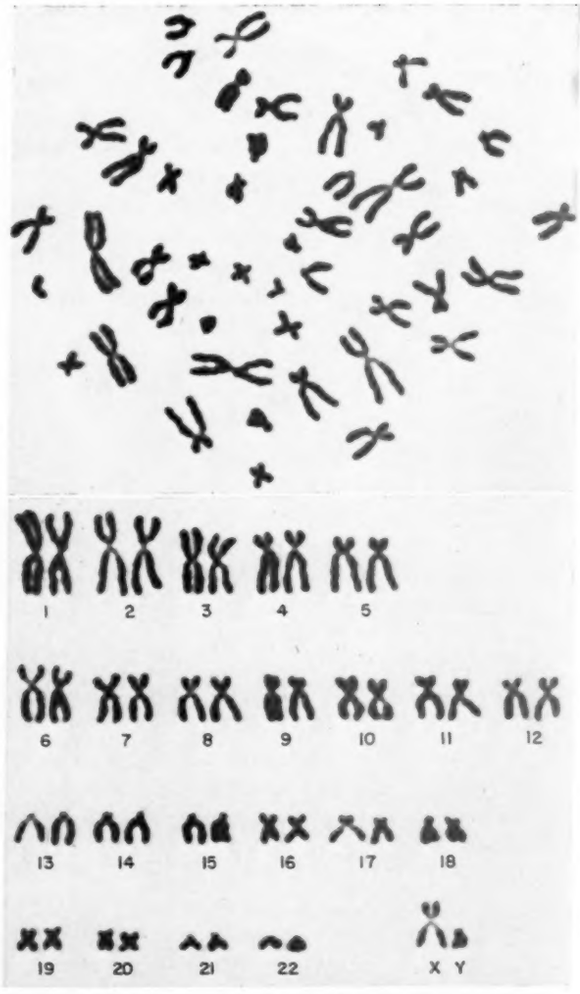


FIG. 4. The chromosome constitution of a normal human male containing twenty-two pairs of autosomes and an XY sex chromosome complex. From: BARR, M. L. and CARR, D. H. *Canad. M. A. J.*, 83: 979, 1960 [*9*].

ference of representative cytologists working in this field was held early in 1960 in Denver, Colorado. Agreement was reached on a standard system for the designation of human mitotic chromosomes ("Denver" international system).

By the Denver system, the pairs of non-sex chromosomes or autosomes are numbered 1 to 22 in descending order of length. The sex chromosomes are referred to as X and Y rather than by a number and are placed near to but separated from the groups of autosomes they resemble. The twenty-two autosome pairs are classified into seven readily distinguishable groups. (Fig. 5.) Within each group the chromosomes are also arranged according to size. The grouping of the chromosomes is further determined by whether the centromere is median, submedian or acro-

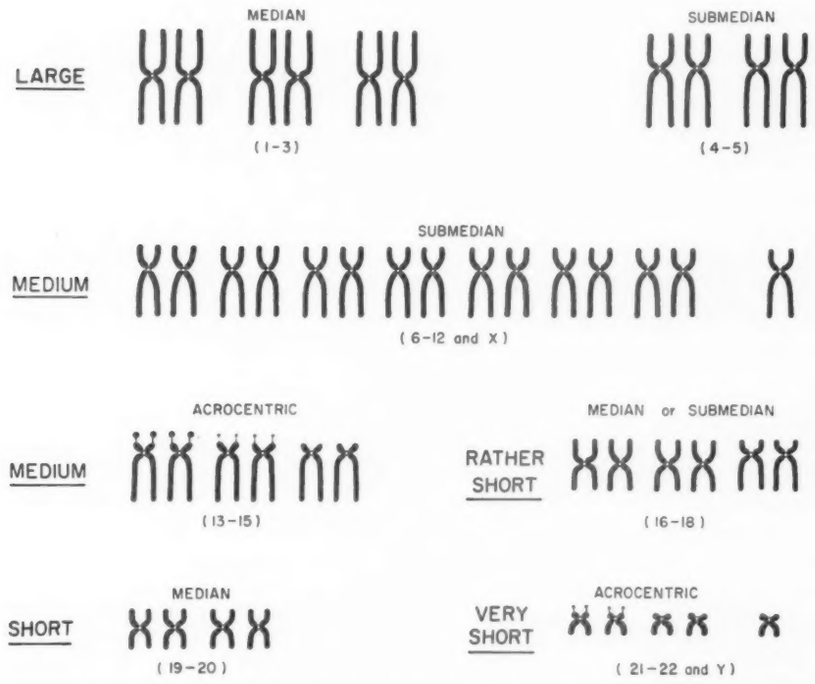


FIG. 5. Idiogram of normal human male with twenty-two pairs of autosomes and an XY sex chromosome constitution. The chromosomes are arranged in seven groups according to their length and centromere position. The classification is in accordance with an international system of nomenclature adopted in Denver, Colorado (see text).

centric (nearly terminal) in location. In the last instance, such chromosomes have a "wish-bone" appearance. In technically optimal preparations a distinction can be made between most if not all chromosomes, although considerable difficulty may be experienced in distinguishing between those of the largest group (6–12, including the X chromosome). The presence of a satellite on the short arm of certain chromosomes (number 13, 14 and 21) assists in the identification of these structures, although there is some disagreement [*101*] as to which chromosomes have these minute structures. The X chromosome resembles the longer chromosomes in group 6–12, especially chromosome 6, from which it is difficult to distinguish. The Y chromosome is similar to the very short acrocentric chromosomes of group 21–22. It has been variously described as being larger [*35,101*] or smaller [*21*] than, or indistinguishable [*37*] from the two smallest autosomes.

The identifying characteristics of the seven groups of human mitotic chromosomes are outlined as follows ("Denver" system):

Group 1–3: *Large* chromosomes with approximately *median* centromeres. The three chromosomes are readily distinguished from each other by size and centromere position.

Group 4–5: *Large* chromosomes with *submedian* centromeres. The two chromosomes are difficult to distinguish, but chromosome 4 is slightly longer.

Group 6–12: *Medium* sized chromosomes with *submedian* centromeres. The X chromosome resembles the longer chromosomes in this group, especially chromosome 6 from which it is difficult to distinguish. This large group is one which presents major difficulty in identification of individual chromosomes.

Group 13–15: *Medium* sized chromosomes with nearly *terminal* centromeres ("acrocentric" chromosomes). Chromosome 13 has prominent *satellites* on the short arms. Chromosome 14 has small satellites on the short arms.

Group 16–18: *Rather short* chromosomes with approximately *median* (in chromosome 16) or *submedian* centromeres.

Group 19–20: *Short* chromosomes with approximately *median* centromeres.

Group 21–22: *Very short*, *acrocentric* chromosomes. Chromosome 21 has *satellites* on its short arms. The Y-chromosome is similar to these chromosomes.

Because of the increasing frequency of reports describing chromosome counts other than the

normal modal number of forty-six, it is important to be aware of the fact that variations in chromosome number are ordinarily encountered in all squash preparations when several nuclei are examined. The significance and statistical distribution of such variations have been studied by Court Brown et al. [*23*] who counted the chromosomes of 1,602 bone marrow cells of sixty-seven individuals with the normal karyotype and the modal number of forty-six. The results indicated that 85 per cent of nuclei contained forty-six chromosomes and that less than forty-four chromosomes were present in approximately 2 per cent, forty-four in 2 per cent, forty-five in 7 per cent, forty-seven in 3 per cent, forty-eight in 0.13 per cent and more than forty-eight in 0.07 per cent. A similar distribution of counts was found in patients with a modal number of forty-five or forty-seven. These variations in chromosome counts are most probably artifacts. However, a significant increase in the percentage of nuclei containing an atypical number (such as forty-five or forty-seven), in the presence of numerous nuclei containing forty-six chromosomes, suggests that the former cells represent genuine aberrations and that two modal numbers are present simultaneously, i.e., that the patient is a mosaic.

At this point it is appropriate to sound a note of caution in the evaluation of recent reports (as well as those which are apt to be forthcoming in rapid order) dealing with human chromosomal analysis. Although technics currently employed yield much more accurate results than those used formerly, they are still lacking in the qualities of precision required for uniform interpretation of results by independent groups of investigators. Moreover, the numerous procedural modifications introduced by different workers, together with the different types of tissue material (bone marrow cells, epidermal cells, leukocytes) studied, create additional factors of variability which leave room for variations in interpretation. This applies particularly to (1) some reported instances of trisomy involving large- or medium-sized chromosomes in which it is difficult to distinguish the X chromosome and to (2) certain morphologically minor chromosomal aberrations involving translocation or partial deletion of a small segment. Lastly, it is important to bear in mind the fact that while the mere counting of the number of chromosomes in a group of nuclei is a relatively simple matter, the matching of homologous pairs, the identification of each individual chromosome, and the interpretation of abnormalities is generally much more difficult and often taxes the skill and ingenuity of experienced chromosomal cytologists. Specific pitfalls and errors inherent in present methods of chromosome identification are discussed in detail by Patau [*101*] who emphasizes the misleading consequences of measuring arm and total lengths of certain individual chromosomes and of attempting to pair off allegedly homologous chromosomes.

GENETIC MECHANISMS CAUSING HUMAN CHROMOSOMAL ABNORMALITIES

Cytogenetic mechanisms responsible for chromosomal aberrations have been conclusively demonstrated in lower forms but not yet in man. Nevertheless, rapidly accumulating casuistic data suggest similar origins for various abnormal human karyotypes. Essentially, the latter consist of deviations from the normal structure and number of chromosomes which frequently result in genic rearrangements and in phenotypic variations. Easily recognizable changes in the structure of chromosomes may be the result of *translocation* or *deletion*. *Inversion* is a type of structural change which is not apparent morphologically, being detected primarily by studies of hereditary transmission. Hence, it is not considered in the present discussion. Variations in the number of chromosomes are usually due to *non-disjunction*.

Human chromosomal aberrations are due either to disturbances in meiotic behavior in the parent (with the formation of abnormal gametes and zygotes) or to mitotic errors during the embryologic development of a normal zygote. In some instances, chromosomal variations may represent the combined result of deviations occurring before and after fertilization.

The following commentary is limited to morphologically demonstrable variations in the normal karyotype and their clinical implications. No attempt is made to deal with the highly speculative consequences in man of those mechanisms concerned with crossing over, duplication, deletion, inversion or linkage of genes.

Translocation. This type of chromosomal aberration results when a chromosome breaks and a fragment becomes attached to another chromosome, often of another pair. When breakage occurs in two chromosomes, the mutual

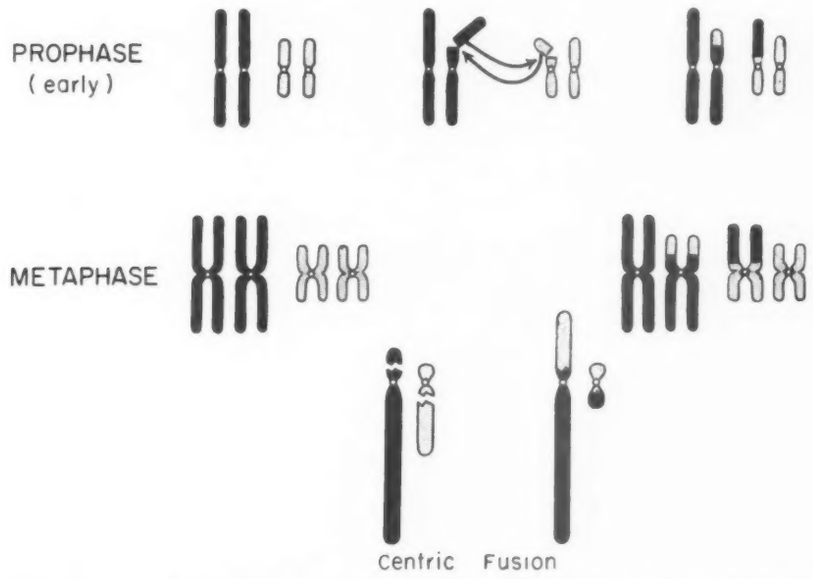


FIG. 6. *Top*, diagram illustrating reciprocal translocation involving the exchange of parts of non-homologous chromosomes early in prophase. (At this stage the chromosomes are actually threadlike, longer and longitudinally reduplicated.) *Middle*, diagrammatic representation showing how the morphology and composition of two pairs of homologous chromosomes at metaphase is altered by reciprocal translocation. The resulting dissimilarity (in size and centromere position) between original members of the two homologous pairs is a potential source of error in the identification of individual chromosomes during karyotype analysis. *Bottom*, schematic illustration of the mechanism of *centric fusion*. This is a special type of reciprocal translocation involving non-homologous acrocentric chromosomes. Breakage close to the centromere occurs in the short arm of one chromosome and in the long arm of the other. Of the two newly produced chromosomes, one is necessarily a minute fragment which is usually lost in a subsequent cell division.

interchange, with reuniting of the fragments, is called *reciprocal* translocation. (Fig. 6.)

Reciprocal translocation, with exchange of parts by two chromosomes belonging to different pairs, differs from crossing over in that the latter involves the exchange of homologous segments of homologous chromosomes. The two new (translocated) chromosomes will survive if each possesses a single centromere since the latter is vital to proper separation during anaphase. If a translocation-produced chromosome lacks a centromere, or if it should have two centromeres, it will be eliminated at a subsequent cell division since proper anaphase separation is impossible.

When translocation occurs in somatic cells during embryogenesis, its effects are incapable of hereditary transmission although they may persist in a clone of cells. Therefore, the presence of a translocation-produced chromosome in the nuclei of different tissues at the time of karyotype analysis presupposes that it was transmitted intact from one of the parents or that it originated during gametogenesis in either parent. An alternative possibility would be the occurrence of translocation during or soon after the first mitotic division of a normal zygote. Under these circumstances survival of the genotypically different daughter cells would result in mosaicism.

It is probable that chromosome translocations too small to be detected by current methods may play a role in the production of certain anomalies in which an abnormal karyotype might be expected but is not found. On the other hand, reciprocal translocation as a causative factor in the production of abnormal human karyotypes has been demonstrated presumptively in some recent studies. These deal principally with (1) certain instances of *mongolism* in which the modal number of chromosomes is forty-six instead of the anticipated number of forty-seven [*20,45,104,108*], (2) the finding of forty-five chromosomes instead of forty-six in some of the phenotypically normal antecedents (carriers) of certain mongols [*20,104*], and (3) a boy with "*polydysspondylism*" whose nuclei contain forty-five instead of forty-six chromosomes [*135*]. As pointed out by Carter et al. [*20*], all the examples of presumed chromosome translocation in man demonstrated thus far involve chromosomes of the acrocentric type.

The finding in these instances of a modal chromosome number which is less than an expected number is probably due to a special type of reciprocal translocation between acrocentric chromosomes, designated *centric fusion* by White [*137*]. This variety of chromosome aberration occurs if each of the involved chromosomes is broken very close to its centromere so that exchange of entire or almost entire chromosome arms takes place ("whole arm" translocation). When breakage occurs in the long limb of one chromosome and in the short limb of the other, transposition of the arms produces a large submedian chromosome and a minute element containing a centromere and a very short region on either side. (Fig. 6.) Since each new chromosome possesses a centromere it is potentially viable. However, the minute fragment (because of its size and predominantly heterochromatic composition) tends to be lost in subsequent divisions. Thus, centric fusion followed by loss of the tiny element leads to a decrease in the basic chromosomal number.

Because of methodologic difficulties in the pairing off of chromosomes during karyotype analysis, it is often quite difficult to decide whether an unmatched chromosome is the result of a translocation or whether it is a trisomic homologue of one of the normal chromosomes. The dissimilarity in size and centromere position between members of the pairs of chromosomes involved in reciprocal translocation (Fig. 6) is a potential source of serious error in the identification of individual chromosomes during karyotype analysis. These and other technical difficulties emphasize the need for restraint of enthusiasm in the interpretation of unusual karyotypes.

Deletion. If, after chromosomal breakage, a fragment fails to translocate to another chromosome and if it lacks a centromere, it will subsequently be lost. The remaining portion of the chromosome survives (because it possesses a centromere) although it is deficient in genes by virtue of those lost in the deleted segment. Having undergone deletion of part of one arm, the involved chromosome is necessarily shorter than its homologue (except in the case of the XY sex chromosomal pair where a partially deleted X chromosome would still be larger than its very short Y homologue).

Partial deletion of a chromosome is often invoked in hypothetic consideration of genetic mechanisms in man [*53,59,126*]. At this writing, only one instance of this type of human chromosomal aberration has actually been demonstrated, at least presumptively. Jacobs and her colleagues [*72*] described a thirty-seven year old woman with primary amenorrhea whose karyotype showed forty-four autosomes and one normal and one abnormal (a short, partially deleted) X chromosome.

Non-disjunction. The most frequent cause of variations in chromosome number is an abnormal movement of chromosomes during nuclear division. (Fig. 7.) Normally, the chromatids (longitudinal halves of a chromosome) separate during mitotic anaphase and the daughter chromosomes migrate to opposite poles of the dividing cell. The same process takes place during anaphase of the second (equational) meiotic division. However, during anaphase of the first or reductional meiotic division, separation occurs between members of each homologous pair rather than between the chromatids of individual chromosomes. Failure of the members of a chromosome pair to separate (disjoin) during anaphase is known as non-disjunction. This phenomenon results in both chromosomes passing into the same nucleus, in which event the other cell receives neither member of the pair. As it is presumed to occur in man, non-disjunction usually involves only one chromosome, although occasionally two are implicated. The resulting individual with an irregular number of chromosomes is an *aneuploid*, having one or two more or less than the basic number of chromosomes, i.e., forty-seven, forty-eight or forty-four, forty-five.

Non-disjunction occurring during maturation of the germ cells causes an extra chromosome to be present in one gamete (which now has the haploid number 23 plus 1) while the other gamete is lacking in this chromosome (having the haploid number of 23 minus 1). When these gametes unite at fertilization with a normal gamete, individuals with forty-seven and forty-five chromosomes, respectively, are formed. The former individual is *trisomic* and the latter is *monosomic* for the particular chromosome involved.

Although there is no direct proof of the existence of meiotic non-disjunction in man, there is little doubt that this mechanism plays a prominent role in the etiology of the majority of types of human chromosomal aberrations reported thus far. The chromosomes involved include the sex chromosomes and certain autosomes. Griboff and Lawrence [*52*] have recently tabulated the

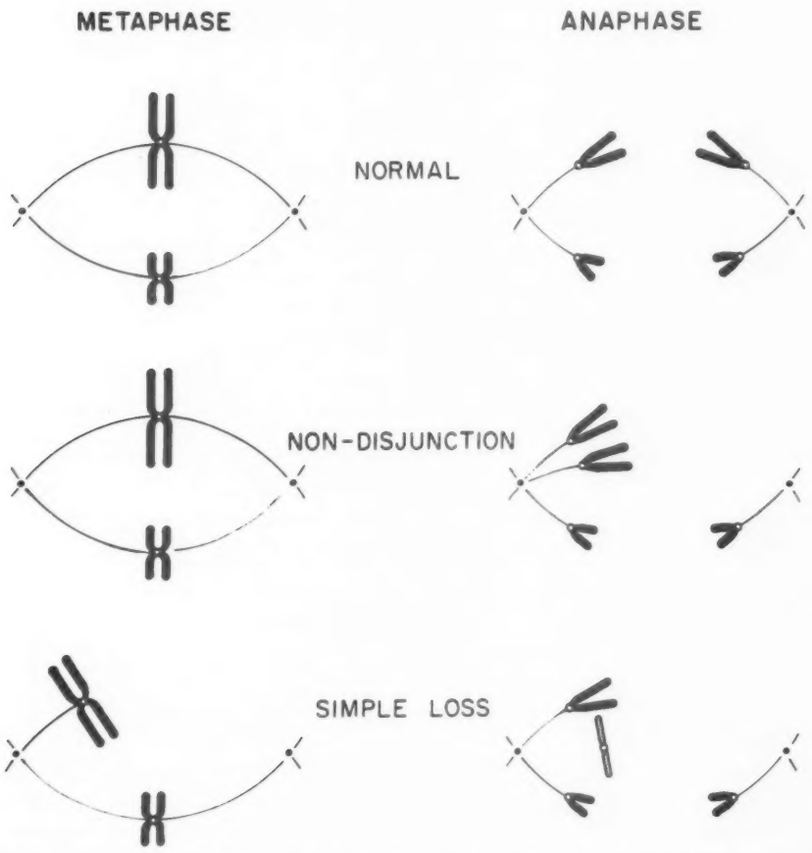


FIG. 7. Chromosome movement in cell division. *Top*, during *normal* metaphase the longitudinally-doubled chromosomes are arranged in the midline equatorial plate. A chromosomal fiber extends in opposite directions from the centromere of each chromosome to the opposing centrioles. In anaphase the halves (chromatids) of each chromosome separate and are now known as daughter chromosomes. They migrate to opposite poles of the cell, presumably being drawn there by their respective centromeres. *Middle, non-disjunction* is characterized by the failure of the daughter chromosomes to separate (disjoin) during anaphase. Instead, both migrate to the same pole of the anaphase nucleus. As a result one daughter cell receives an extra chromosome while the other cell becomes deficient in this chromosome. *Bottom, simple loss* of a chromosome is sometimes due to failure of a metaphase chromosome to become oriented on the equatorial plate. Apparently, the centromere of one but not of the other member of a pair of chromatids is effective in drawing its daughter chromosome toward the pole of the anaphase nucleus. The daughter chromosome with the inactive centromere fails to migrate to the nucleus of either daughter cell. It remains in the cytoplasm where it eventually disintegrates.

numerous theoretic sex chromosome combinations in the zygote which may result from meiotic non-disjunction.

While non-disjunction may occur in somatic cells, its effects are not transmissible to succeeding generations and therefore are of little interest from the point of view of heredity. However, the occurrence of somatic non-disjunction during the very early mitotic divisions of a zygote explains certain sex-chromosomal abnormalities in man (see discussions of *mosaicism* and *sexual anomalies*).

Simple Loss of a Chromosome. Another type of abnormal chromosome movement during cell division which causes variations in chromosome number is one which occasionally results from failure, in the metaphase, of a chromosome to become oriented on the equatorial plate. (Fig. 7.) In this event the centromere of one, but not of the other, member of a pair of chromatids is effective in drawing the chromatid toward the pole of the anaphase nucleus. As a consequence of this type of mitotic spindle accident the chromosome with the inactive centromere fails to migrate to the nucleus of either daughter cell. It is thus apparent that while the two daughter cells after non-disjunction each have an abnormal number of chromosomes, only one of the two daughter cells after simple loss of a chromosome has an abnormal number.

TABLE 1
REPORTED INSTANCES OF HUMAN SEX MOSAICISM

Karyotype	Tissue Cells	Clinical Conditions	Sex Chromatin	Reference
(45/46) XO/XX	Marrow	Turner's syndrome	+	36
(45/46) XO/XX	Marrow*	Turner's syndrome	−	42
(45/46) XO/XX	Marrow	Turner's syndrome	−	114
(46/47) XX/XXY	Marrow	Klinefelter's syndrome	+	41
(46/47) XX/XXY	Marrow	Klinefelter's syndrome	+†	24
(46/47) XY/XXY	Marrow	Klinefelter's syndrome	−	114
(45/46) XO/XY	Marrow‡	Intersex (true hermaphrodite ?)	−	63; 64
(45/47) XO/XXX	Skin, peripheral blood§	Phenotypic female, eunuchoid, primary amenorrhea, absent vagina, no "drumsticks"	+†	72
(48/49) XXXY/XXXXY	Marrow	Phenotypic male, eunuchoid, very small testes, mental retardation	+‖	70

* Skin culture exhibited (45) XO with no evidence of mosaicism.
† Double sex chromatin bodies present in some nuclei.
‡ Skin culture exhibited (46) XY with no evidence of mosaicism.
§ Sternal marrow yielded (45) XO with no evidence of mosaicism.
‖ Double and triple sex chromatin bodies present in some nuclei.

Mosaicism. The presence of genetically dissimilar cells in adjacent tissues of an individual is known as mosaicism. When present in plants (usually as a result of a graft) this condition is referred to as a *chimera*. Thus far, two forms of mosaicism have been recognized in man. The first, termed *erythrocyte mosaicism* [*78*], occurs in fraternal twins and is characterized by the presence of two distinct blood groups (A and O) in one individual. The finding of "drumsticks" (indicative of a female genotype) in the leukocytes of the male twin in two cases [*17,96*] strongly suggests that white as well as red blood cells may participate in this type of mosaicism. In this event the condition would be more properly designated as a *blood mosaic* or *chimera*. This is a rare condition and chromosome analyses in affected persons have not yet been reported. The second is a heterogeneous group of patients with anomalous sexual development in whom somatic cells contain sex chromosome complements of more than one type (*sex mosaicism*).

The occurrence of mosaicism means that the body contains two or more stem-lines of cells with different chromosome numbers or constitution. In other words, the genetically dissimilar cells are the progeny of different ancestral cells which have developed along separate stem-lines. To date there are no reports of more than two cell types in human sex mosaicism. The relatively few instances which have been published indicate that the normal XX or XY sex chromosome complement is usually, although not necessarily, a component of the mosaic. (Table 1.)

Sex mosaicism has been found (see Table 1 for references) in Turner's syndrome showing a (45/46) XO/XX karyotype, in Klinefelter's syndrome with (46/47) XX/XXY or XY/XXY complements, and in an intersex, possibly a true hermaphrodite, whose chromosomal constitution was (45/46) XO/XY. A (45/47) XO/XXX combination was found in a phenotypic female with eunuchoidism, primary amenorrhea, absent vagina and low normal intelligence. There were several pigmented naevi, but no other stigmata of Turner's syndrome. In the absence of operative and gonadal histologic findings it is not possible to assess the particular type of gonadal dysgenesis present in this patient. A (48/49) XXXY/XXXXY mosaic has been found in a phenotypic male with mental retardation.

It should be understood that the demonstration of human sex mosaicism is still in its infancy. Further technical refinements are required to eliminate the occurrence of artifacts and other sources of error in the counting and identification of individual chromosomes. Several of the reported studies indicate that the interpretation rendered has been presumptive, although there is little doubt that true mosaicism does exist.

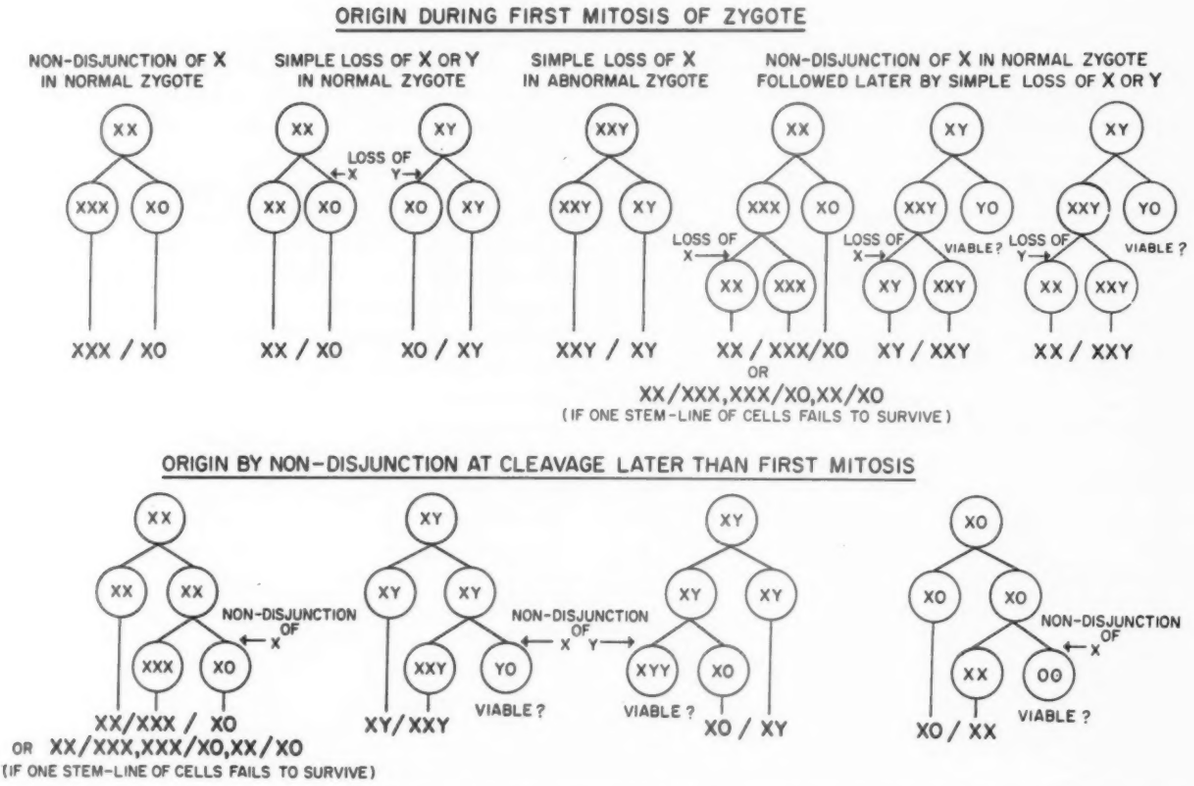

ORIGIN DURING FIRST MITOSIS OF ZYGOTE
NON-DISJUNCTION OF X IN NORMAL ZYGOTE
SIMPLE LOSS OF X OR Y IN NORMAL ZYGOTE
SIMPLE LOSS OF X IN ABNORMAL ZYGOTE
NON-DISJUNCTION OF X IN NORMAL ZYGOTE FOLLOWED LATER BY SIMPLE LOSS OF X OR Y
LOSS OF X
LOSS OF Y
VIABLE?
XXX / XO
XX / XO
XO / XY
XXY / XY
XX / XXX/XO
XY / XXY
XX / XXY
OR
XX/XXX,XXX/XO,XX/XO
(IF ONE STEM-LINE OF CELLS FAILS TO SURVIVE)
ORIGIN BY NON-DISJUNCTION AT CLEAVAGE LATER THAN FIRST MITOSIS
NON-DISJUNCTION OF X
NON-DISJUNCTION OF X Y
NON-DISJUNCTION OF X
XX/XXX / XO
OR XX/XXX,XXX/XO,XX/XO
(IF ONE STEM-LINE OF CELLS FAILS TO SURVIVE)
XY/XXY
VIABLE?
VIABLE?
XO / XY
XO / XX
VIABLE?

FIG. 8. Schema illustrating various (but not all) mechanisms by which sex mosaicism may be produced. In general the latter is due to divisional errors such as non-disjunction or simple loss of an X or Y chromosome, occurring singly or in sequence. These errors may take place during the very first mitosis of a zygote or at a later cleavage division of the developing embryo or during both. The original zygote may have a normal or abnormal sex chromosome constitution.

Indeed, it is a fair prediction that additional instances will be uncovered when chromosomal analysis is performed concurrently in multiple types of tissue material. This is illustrated in the (45/47) XO/XXX case in which mosaicism was present in cells of the skin and peripheral blood, but not in bone marrow cells. According to the authors [*72*], this discrepancy is best explained by the likelihood that the marrow may consist only of cells of one type (forty-five chromosomes) while other tissues including lymph nodes and other sites in the reticuloendothelial system contain cells of both types (forty-five and forty-seven chromosomes). A comparable but reversed situation has been noted (1) in a patient with Turner's syndrome who possessed a (45/46) XO/XX mosaic constitution in the marrow cells but only a single (45) XO condition in the skin [*42*], and (2) in an intersex who may be a true hermaphrodite with mosaicism in the marrow cells but a normal (46) XY state in the skin [*63,64*].

Because of the variability of chromosomal counts in normal persons, Court Brown et al. [*23*] have emphasized the difficulties in proving that a particular count indicates a mosaic. Accordingly, these investigators have formulated certain criteria requiring fulfillment before the diagnosis can be established. Essentially, these include ascertaining that (1) the number of cells with abnormal counts is not ascribable to chance, (2) the excess of atypical cells can be demonstrated in more than one examination and preferably in more than one tissue, and (3) the same karyotype is present in each of the deviant cells of the respective stem-lines.

The developmental pathways leading to sex mosaicism are fairly numerous. (Fig. 8.) In general these have their origin in abnormal sex chromosome behavior occurring (1) during cell division before the zygote is formed, i.e., in gametogenesis; (2) during the first mitotic division of a zygote; or (3) at a cleavage of a developing ovum later than the first mitosis. In some instances, particularly when XXY is present as one of the components of the mosaic,

it is necessary for at least two separate divisional errors to have occurred.

Non-disjunction and simple loss of a chromosome are the divisional errors which satisfactorily explain all types of sex mosaicism. The composition of a mosaic depends upon the type of divisional error and upon the stage of development at which the aberration occurred. Moreover, it is theoretically possible that different pathways of development can lead to the same type of mosaic. For example, in the cases previously mentioned, Figure 8 indicates how the (45/46) XO/XX karyotype in Turner's syndrome may result from simple loss of one daughter X chromosome during the first mitotic division of a normal XX zygote or how it could be produced in an abnormal XO embryo by the non-disjunctional gain of an X chromosome during a cleavage later than the first division. The (46/47) XX/XXY mosaic is probably caused by X chromosome non-disjunction during the first mitosis of an XY zygote with the formation of an XXY and a YO cell. The latter is generally believed to be non-viable. The XXY cell subsequently undergoes simple loss of a Y chromosome, which produces two stem-lines of cells, XX and XXY. Reference to Figure 8 indicates the several ways in which the XY/XXY combination can result. The (45/46) XO/XY intersex could be produced by simple loss of a daughter Y chromosome during the first mitosis in a normal XY zygote while the (45/47) XO/XXX phenotypically female eunuchoid probably is the result of non-disjunction of a daughter X chromosome during the first mitosis of a normal XX zygote. The (48/49) XXXY/XXXXY mosaic is due to a series of divisional errors, some of which may occur during gametogenesis in either or both parents, the remainder taking place early in the course of embryonic development.

A triple mosaic has not been reported although its occurrence is not impossible. Indeed, it is quite conceivable that certain mosaics with only two demonstrable cell types may actually have originated with three stem-lines of cells, one of which failed to survive. For obvious reasons the development of a triple stem-line mosaic requires the occurrence of an error at a division later than the first mitosis.

KARYOTYPES IN VARIOUS MORBID STATES

It should be clearly understood in the following discussion that many of the recently reported chromosomal aberrations are classified presumptively rather than definitively by the respective authors. Indeed, some of the karyotypes presented, on the basis of published photomicrographs, are susceptible to more than one interpretation. Hence, until considerably more data are accumulated precise characterization of an unspecified number of reported chromosomal abnormalities must be regarded as unsettled while their proffered designation is accepted as tentative.

On the other hand, the finding of a normal chromosome constitution in a given clinical disorder does not exclude the possibility that a chromosomal aberration may nevertheless exist. Indeed, a variety of chromosomal anomalies, non-detectable by current technics, may be masked by an apparently normal karyotype.

In general the reported instances of abnormal chromosomal complexes in man are characterized by involvement of sex chromosomes or of autosomes, and seldom of both. The fact that there are presently more examples of sex chromosome anomalies than of those dealing with autosomes is probably due to a greater concentration of attention on patients with sexual disorders.

The principal disorders in which chromosomes have been analyzed may be grouped as follows: (1) a rather large variety of sexual anomalies, (2) individuals, usually oligophrenic, with more than two X chromosomes, (3) mongolism, (4) an heterogenous assortment of conditions exhibiting multiple congenital anomalies, and (5) leukemia. The chromosome findings are tabulated separately for most of the different types of disorders. A summarizing compilation for the entire group is presented at the end of the discussion in Table IX.

It will be evident that in the majority of instances there is no close correlation between karyotypes and disease patterns. Although pathogenetic mechanisms are properly invoked to explain most of the demonstrated chromosome abnormalities, it must be emphasized that precise etiologic factors are seldom ascertainable.

Sexual Anomalies. In itself, karyotype analysis in individuals with congenital errors of sexual development is of great interest, but in addition it has shed new light on the derivation of the nuclear sex-chromatin body of Barr. In fact, the discovery of discordances between nuclear and phenotypic sex, i.e., chromatin-positive Klinefelter's syndrome and chromatin-negative Tur-

ner's syndrome and testicular feminization, provided an early impetus to application of the newer technics for chromosome study to clinical problems of aberrant sexual development. Data yielded by complementary karyotype and sex chromatin studies bring us ever closer to the ultimate elucidation of the pathogenesis of various poorly understood sexual disturbances.

Pseudohermaphroditism: In this type of abnormal sexual development, elements of both sexes are present in the genital tract but the gonads are either testes or ovaries. In the former instance the condition is referred to as male, and in the latter as female, pseudohermaphroditism. In either case the nuclear sex chromatin pattern is concordant with the gonadal sex. Similarly, the sex chromosome complement appears to correspond to the type of gonads present. In both types the modal chromosome number is the normal forty-six, the XY complex being present in male pseudohermaphrodites [*21,67,110*] and the XX in the female variety [*1,132*].

A special variety of male pseudohermaphroditism, known as *testicular feminization*, is of particular interest because these individuals are phenotypic females who often escape detection until they are well on in adult life. Despite the fact that they have undescended testes and primary amenorrhea, the external genitalia are female, there is normal breast development, a feminine habitus (except for scant or absent pubic and axillary hair) and they are capable of sexual intercourse and orgasm. The fact that the chromatin-negative nuclear sex is at variance with the phenotypic sex is the reason why the sex chromatin pattern should be determined routinely in all women with primary amenorrhea.

The absence of a demonstrable gross chromosomal aberration in the instances of pseudohermaphroditism thus far reported does not exclude the likelihood that some such mechanism does nevertheless exist. Indeed, it is probable that gene mutations are primary causes in most if not all cases, including those due to congenital adrenocortical hyperfunction. Analysis of familial cases of testicular feminization, which is transmitted through the maternal line and occurs only in offspring with testes, suggests that the condition is associated with or caused by a sex-linked recessive or a sex-limited autosomal dominant gene [*13,54,67,105*]. A rationale favoring the latter notion has been adduced recently by Puck, Robinson and Tjio [*110*]. Their findings in the female transmitters of the defect in the proband studied by them indicated that all exhibited a delayed menarche of approximately eight years. It would appear, at least in one pedigree in which the details of the menstrual history of females in previous successive generations were specifically sought, that the presence of the responsible gene in heterozygous condition in an XX individual causes a delay of menarche in otherwise healthy women.

TABLE II
REPORTED KARYOTYPES IN INDIVIDUAL CASES OF TRUE HERMAPHRODITISM

Karyotype	Tissue Cells	Sex Chromatin	Reference
(46) XX	Circulating leukocytes	+	65
(46) XX	Skin and marrow	+	57
(46) XX	Marrow	+	35
(46) XX	Marrow	+	35
(46) XX	Marrow	+	25
(46) XX	Marrow	+	49
(46) XX	Skin	+	55
(46) XY	Skin	−	55
(45/46) XO/XY (46) XY	Marrow Skin	−	63; 64*
(46) XY	Marrow	−	114
(46) XX	Marrow	n.t.†	115
XXY‡	Testis	+	51

* Gonadal biopsy compatible with but not conclusive for true hermaphroditism.

† Not tested.

‡ Examination of uncultured, fixed gonadal biopsy specimen. Chromosomes were "analyzed" but not counted.

True hermaphroditism: This type of intersex possesses male and female elements not only in the genital tract but in the gonadal tissue as well. Such individuals have testes and ovaries or ovotestes with or without a testis or an ovary. Approximately two-thirds of twenty-five patients studied are chromatin-positive and one-third is chromatin-negative [*54*]. At this writing the results of chromosome analysis in ten proved true hermaphrodites have been published. (Table II.) Another intersex, originally reported as a true hermaphrodite [*63*], is added tentatively to this group. In a subsequent communication, Hirschhorn et al. [*64*] found that the gonadal biopsy histology was suggestive of, but apparently not conclusive for, the diagnosis of true hermaphroditism. (From the published description and photomicrographs, the pathologic find-

TABLE III
REPORTED KARYOTYPES IN KLINEFELTER'S SYNDROME

Karyotype	Sex Chromatin	Reference
(47) XXY	+	74;39;97*
(48) XXY†	+	39;84;85
(47/46) XXY/XX	+	41; 24*a*‡
(48) XXYY	+	95
(46) XY	−	41
(47/46) XXY/XY	−	114

* In addition to the characteristic (47) XXY karyotype, these workers noted in the majority of cases the presence of significant numbers of cells with forty-six and forty-eight chromosomes.

† Patients with mongolism and an extra chromosome number 21.

‡ Double sex chromatin masses in some nuclei of this patient.

ings are clearly those of a dysgenetic gonad and only time will tell whether this phenotypically female infant has true hermaphroditism or an atypical form of gonadal dysgenesis.) An additional report by Greene et al. [*51*] in 1954 is of uncertain significance since the examination was made of fixed uncultured testicular material (the sex chromosomal constitution was interpreted as XXY). Of the ten unquestionable true hermaphrodites, nuclear sex was determined in nine. Seven of these were chromatin-positive. Each showed the karyotype of the normal human female, a total of forty-six chromosomes with an XX sex chromosome constitution. The remaining two subjects were chromatin-negative, each exhibiting the chromosome composition of a normal human male, (46) XY. The intersex who "may be" [*64*] a true hermaphrodite was chromatin-negative and showed a (45/46) XO/XY mosaic chromosome constitution.

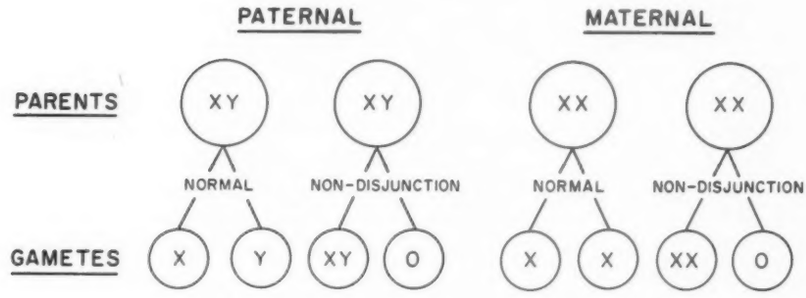
The occurrence of sex mosaicism in true hermaphroditism would not be surprising. Indeed, one might expect to encounter it in individuals possessing gonadal tissue of both sexes. The fact that it has been found only once, and in this instance in a patient who is not unequivocally a true hermaphrodite, may simply be due to the small number of patients subjected to analysis, or to restriction of the examination to only one type of tissue in most instances. In fact, this single instance of mosaicism [*64*] would have eluded detection had the skin alone been examined, for the karyotype in the dermal cells was (46) XY. Another explanation for the apparent infrequency of mosaics in this group of disorders is the possibility that one or two ancestral stem-lines of cells may have failed to survive.

As in pseudohermaphroditism, the absence of an obvious chromosomal anomaly in true hermaphroditism does not eliminate the possibility of its existence, particularly in the form of small translocations or deletions. It is virtually impossible to assess factually the role of genic factors in view of the sterility of these patients.

Although chromosome analysis has not yet elucidated the pathogenesis of this disorder, the fact that testicular tissue is present in the absence of a Y chromosome is of considerable interest. As pointed out by Ferguson-Smith and Johnston [*33*], this finding suggests that the normal Y chromosome is not the only important factor in the initiation of male differentiation.

Klinefelter's syndrome: Individuals with this disorder are phenotypic males with anatomic derangement (sclerosing tubular fibrosis, seminiferous tubular dysgenesis, microorchidism) and functional impairment (sterility, androgen deficiency) of their testes. Approximately half of the patients are chromatin-positive. This group constitutes the most frequent type of human sex anomaly.

The chromosome constitution in the majority of the chromatin-positive patients is (47) XXY. (Table III.) There is no positive evidence to indicate whether the extra chromosome is the X or Y chromosome, i.e., whether such patients are chromosomal males with an extra X chromosome or females with an extra Y chromosome. A presumptive (46/47) XX/XXY mosaic has been described by Ford et al. [*41*] and by Crooke and Hayward [*24*] in two chromatin-positive persons with Klinefelter's syndrome. For some unclear reason, several of the chromatin-positive nuclei in one of these patients [*24*] contained double sex-chromatin bodies. Mosaicism has also been noted by Nowakowski et al. [*97*] who performed chromosome analysis in thirteen individuals with chromatin-positive Klinefelter's syndrome. While these investigators confirmed the characteristic (47) XXY karyotype, they noted in addition that the majority of subjects also had significant numbers of cells with forty-six and forty-eight chromosomes. A count of forty-eight chromosomes with an XXYY sex chromosome complement has been reported recently by Muldal and Ockey [*95*] in a chromatin-positive individual with Klinefelter's syndrome. In this instance the two extra chro-



ZYGOTES: SPERM \ OVUM	X	XX	O
X	XX NORMAL FEMALE	XXX TRIPLE X FEMALE	XO TURNER'S SYNDROME
Y	XY NORMAL MALE	XXY KLINEFELTER'S SYNDROME	YO VIABLE ?
XY	XXY KLINEFELTER'S SYNDROME	XXXY TRIPLE X MALE	XY NORMAL MALE
O	XO TURNER'S SYNDROME	XX NORMAL FEMALE	OO VIABLE ?

FIG. 9. Diagrammatic illustration of the effects of non-disjunction occurring during gametogenesis in either or both sexes. The various zygotic possibilities, indicated in the squares, show how (1) Klinefelter's and Turner's syndrome may result from non-disjunction during spermatogenesis or oögenesis, (2) the triple X female can be the product of non-disjunction occurring only during oögenesis and (3) the triple X male may be due to non-disjunction in both parents.

mosomes are interpreted as an X and a Y. Very few karyotype analyses have been reported in chromatin-negative Klinefelter's syndrome. One [*41*] showed the chromosome constitution of a normal male, (46) XY, while another [*114*] revealed a presumptive (46/47) XY/XXY mosaic.

It is possible that two chromatin-positive cases reported by Ferguson-Smith [*34*] and by Barr et al. [*6,10*] with a (48) XXXY karyotype belong in the category of Klinefelter's syndrome. The dominant clinical feature of each patient was severe mental deficiency and one, in addition, had congenital malformations. These are discussed under "Poly-X Conditions."

The fact that at least five different karyotypes have been encountered in a relatively small number of patients with Klinefelter's syndrome opens the door to many questions. Final answers must await the accumulation of more data. From present indications it appears that Klinefelter's syndrome is not a homogeneous entity and that its clinicopathologic features may be produced or simulated by a variety of pathogenetic mechanisms. In this connection a note of caution should be sounded against making this diagnosis merely because a chromatin-positive phenotypic male, especially a child with congenital anomalies, happens to have an XXY sex-chromosome constitution. (Table VIII.)

The karyotype most commonly encountered in Klinefelter's syndrome, (47) XXY, is apparently the result of non-disjunction of the sex chromosomes during gametogenesis of either parent. (Fig. 9.) For example, non-disjunction during oögenesis produces an ovum with two X chromosomes and one without a sex chromosome. Then, fertilization of the XX ovum by a normal Y-bearing sperm forms an XXY zygote, the progenitor of Klinefelter's syndrome. Non-disjunction during spermatogenesis results in one sperm having an XY combination and the other having no sex chromosome. When the XY sperm fertilizes a normal X-containing ovum an XXY zygote is produced. There is some reason to believe that meiotic non-disjunction as an

TABLE IV
KARYOTYPES FOUND IN TURNER'S SYNDROME

Karyotype	Sex Chromatin	Reference
(45) XO	−	40;73;44
(45) XO	+	55;32
(46) XX	+	32*
(45/46) XO/XX	−	42;114
(45/46) XO/XX	+	36

* Two patients, one of whom showed a translocation on one of the X chromosomes causing it to correspond in size to chromosome number 3.

etiologic factor in Klinefelter's syndrome is more likely in the mother than in the father [*33*]. The possible pathogenetic mechanisms involved in the production of the reported sex mosaics are outlined in Figure 8.

Turner's Syndrome: Individuals with this type of anomaly are phenotypic females with normal but underdeveloped female genitalia and absence of secondary sexual characteristics at puberty. In addition, they have an increased urinary excretion of gonadotropins and usually exhibit one or more associated congenital abnormalities which characteristically consist of shortness of stature, webbing of the neck, broad shield-like chest and cubitus valgus among others. The gonads usually are represented by vestigial fibrous streaks and the gonadal defect itself is but one of the particular constellation of congenital anomalies that is designated Turner's syndrome.* Approximately 80 per cent of the patients are chromatin-negative.

The karyotype in the majority of individuals with chromatin-negative Turner's syndrome is neither male nor female. It is composed of forty-five chromosomes with an XO sex chromosome complement (Table IV). The same (45) XO constitution has been found in some chromatin-positive patients by Grumbach et al. [*55*] and by Ferguson-Smith [*32*]. In two other chromatin-positive patients, Ferguson-Smith [*32*] demonstrated a (46) XX constitution. In one of these patients there was a translocation on one of the X chromosomes causing it to correspond in size to chromosome number 3. Ford and his group [*36*] found a (45/46) mosaic in one chromatin-negative and in two chromatin-positive subjects. Detailed analysis of the cells of one of the chromatin-positive patients indicated the presence of a sex mosaic, XO/XX. A similar presumptive (45/46) XO/XX mosaic karyotype was found in chromatin-negative patients with Turner's syndrome by Fraccaro et al. [*42*] and by Sandberg et al. [*114*].

Because of the frequently associated congenital anomalies, it is reasonable to expect that chromosome analysis of more patients will uncover additional varieties of abnormal karyotypes, especially those involving autosomes. Such findings would be comparable to those encountered in patients with Klinefelter's syndrome who are also mongoloid. As already mentioned, these (48) XXY individuals have combined aberrations involving the sex chromosomes and the autosomes. Examples of autosomal anomalies to be anticipated in certain patients with Turner's syndrome will be cited under "Multiple Congenital Anomalies."

That other types of chromosomal anomalies, involving the sex chromosomes, may exist is suggested by a preliminary communication of Fraccaro et al. [*43*]. In two chromatin-positive patients these investigators found a modal number of forty-six chromosomes. There was only one X chromosome but another unmatched, long metacentric chromosome may have been the other X chromosome in an abnormal form.

In Turner's syndrome, as in Klinefelter's syndrome, and for the same reasons, the condition appears to be an heterogeneous one. This is consistent with the variability of the clinicopathologic features of both syndromes which in turn bespeaks a corresponding variability in pathogenetic mechanisms. Nevertheless, it is most probable that non-disjunction during spermatogenesis or oögenesis is the error which accounts for the anomalous (45) XO karyotype found in most of these patients. (Fig. 9.) As a result of non-disjunction on the paternal side, an XY sperm and an O sperm are produced. Fertilization of a normal X-containing ovum by the latter results in an XO embryo with a potential for Turner's syndrome. When non-

* Although the gonadal lesion is an important diagnostic feature of Turner's syndrome, there are other and often unrelated forms of gonadal dysgenesis which differ from the type encountered here. In fact, gonadal dysgenesis is really a generic disorder of the gonads embracing a broad spectrum of developmental anomalies. These are characterized by partial or defective development of various ovarian or testicular elements or of both. Among the better known examples are those found in male pseudohermaphroditism, true hermaphroditism, Klinefelter's syndrome and testicular germinal aplasia. Certain unclassified instances are illustrated under the heading "Miscellaneous."

disjunction occurs during maternal gametogenesis both X chromosomes pass to one ovum while the other receives none. When the latter (O ovum) is fertilized by a normal X-bearing sperm an XO zygote is produced. Possible mechanisms in the formation of the reported sex mosaics are outlined in Figure 8.

Since the pathogenetic mechanism (meiotic non-disjunction) is presumably the same for both Klinefelter's syndrome and Turner's syndrome, XXY and XO zygotes should occur with equal frequency. However, the incidence of XXY-Klinefelter's syndrome is considerably greater than that of XO-Turner's syndrome. In a consideration of this discrepancy Ford [*36*] suggests certain possible explanations, including differential mortality of the zygotes and differential fertilizing capacity of the alternate types of sperm.

Miscellaneous: Chromosome analysis has been performed in a few other types of sexual disorders which, because of insufficient data or clinicopathologic atypicity, cannot be classified definitely. Nevertheless, the findings are of considerable interest in that they may eventually supply important missing links.

Abnormal X-chromosome and gonadal dysgenesis: Jacobs and her associates [*72*] described a thirty-seven year old woman with primary amenorrhea and absence of secondary sexual characteristics. Repeated buccal smears showed sex chromatin in only 7 per cent of the cells and no "drumsticks" were found in 1,000 leukocytes. The sex chromatin bodies were "smaller than usual." There was no shortness of stature or other stigmata of Turner's syndrome. At laparotomy the gonads were noted to be streaks of rudimentary tissue. Histologic section showed ovarian tissue with a few primitive follicles in one gonad but the tissue in the other gonad was not recognizably ovarian. Chromosome analysis, performed on sternal marrow, peripheral blood, skin, and material from both gonads, uniformly yielded a modal chromosome number of forty-six. The sex chromosome constitution was interpreted as being composed of one normal X chromosome and one abnormal (short) X chromosome. The latter abnormality is regarded as the result of partial deletion of a segment of the chromosome. This aberration is thought to have occurred during gametogenesis in either parent.

The karyotype in this patient permits an alternative interpretation which was considered by the authors but dismissed for several reasons. While the latter appeared to be valid at the time, they are not irrefutable in the light of subsequent developments in the rapidly expanding field of human chromosome analysis. It is therefore possible that the unmatched pair of chromosomes does not represent one normal and one partially deleted X chromosome, but rather the chromosome thought to be an abnormal (short) X chromosome might actually be an extra autosome similar to those of the sixteenth pair. In this event the patient would be classified as having an XO sex chromosome constitution with autosomal trisomy.

The fact that the nuclear sex chromatin of this patient was smaller than usual seems to be consistent with partial deletion of one of the X chromosomes. However, the presence of "small" sex chromatin bodies in a given patient does not necessarily imply a loss of some X chromosome material since Sohval and Casselman [*120*] have shown that temporary reduction in the size of the nuclear sex chromatin can result from extraneous influences.

Reference was made previously to a brief report by Fraccaro et al. [*43*] of unusual chromosome findings in chromatin-positive patients with Turner's syndrome. These patients also had "streak" gonads with a modal number of forty-six chromosomes but only one normal X chromosome. The authors could not decide whether the unmatched large median chromosome is an *extra autosome* corresponding to those of pair number 3 (trisomy) or whether it is an *abnormal X chromosome*. In the latter case it could represent an isochromosome derived from the long arm of an X chromosome or it could be the result of duplication or translocation. The chromosome abnormality appears to be similar to that encountered in a chromatin-positive patient with Turner's syndrome (Table IV) by Ferguson-Smith [*32*].

"Pure" gonadal dysgenesis: Harnden and Stewart [*59*] reported a (46) XY chromosome constitution in a nineteen year old, tall, eunuchoid, phenotypic girl with primary amenorrhea, increased urinary gonadotropin excretion, chromatin-negative oral smear nuclei and no "drumsticks" in the circulating leukocytes. Withdrawal bleeding after estrogen administration indicated the presence of competent uterine endometrium. It was presumed by the authors that ovaries were absent as a result of defective development (gonadal dysgenesis). The term

pure alludes to the absence of associated developmental anomalies. It is to be noted, however, that without access to operative and histologic information concerning the gonads it is impossible to eliminate male pseudohermaphroditism or even true hermaphroditism as diagnostic possibilities. Although the reported karyotype in this case is that of a normal male the possibility of a chromosomal translocation or of a deletion too small to be detected could not be excluded by the authors.

A thirty-eight year old, six feet tall, phenotypic woman mentioned by Sandberg et al. [*114*] is also of interest in this connection. Although the buccal mucosa was chromatin-negative the circulating leukocytes contained "drumsticks." Chromosome analysis of thirty bone marrow metaphase nuclei indicated that 10 per cent had forty-five chromosomes, 70 per cent had forty-six, and 13 per cent had forty-seven. It is the authors' interpretation that while only 10 per cent of the cells contained forty-five chromosomes, the patient has an XO constitution, the modal number of forty-six resulting not from XX but from trisomy of one of the two largest autosomes. Because of the XO sex chromosome complement this woman was regarded as having Turner's syndrome. While some type of gonadal dysgenesis is probably present in this patient, much more data are needed to clarify its nature. The mere presence of a modal number of forty-five chromosomes with an XO sex chromosome constitution, even in a chromatin-negative, short woman without "drumsticks" in the peripheral leukocytes, does not necessarily indicate that such a person has Turner's syndrome. Fertility in such a person has been reported by Bahner et al. [*2*], indicating that gonadal dysgenesis here cannot be significant, if present at all.

"*Atypical*" *gonadal dysgenesis:* Ferguson-Smith and Johnston [*33*] refer briefly to a chromatin-negative child reared as a male who had a uterus and fallopian tubes with a testis on one side and a rudimentary gonadal "streak" on the other. The karyotype was interpreted as (45) XO. This type of sexual anomaly cannot be classified definitively as one of the conventional disorders such as male pseudohermaphroditism or true hermaphroditism even though pathologic and chromosomal data are available. Apparently it belongs to an unusual variety of gonadal dysgenesis which may represent a transition form between some of the better known anomalies.

A similar case was reported by Bloise et al. [*12*]. This also concerned a chromatin-negative child reared as a male. Here, too, there was a uterus with a testis near the fallopian tube on one side. On the opposite side there was "connective tissue as seen in gonadal dysgenesis with negative chromatin pattern." As in the previous case, the karyotype was interpreted as (45) XO. The authors regarded this patient as having "Turner's syndrome with male phenotype." However, the eponymic designation "Turner's syndrome" applies only to phenotypic females and does not include individuals with well organized testicular structures. Although one may conceive of a male counterpart of Turner's syndrome [*122b*], it seems more appropriate at this time to classify this patient tentatively in the general category of "atypical" gonadal dysgenesis.

Poly-X Conditions. Reports of individuals who are polysomic for the X chromosome, i.e., more than two X chromosomes,* are appearing with increasing frequency. These are enumerated in Table v. The most frequent chromosomal constitution in this category is represented by forty-four autosomes plus XXX with a total count of forty-seven in phenotypic females. The first such patient to be described [*68*] was the now well known, but unfortunately named, twenty-eight year old "Superfemale" with hypoplastic atrophic ovaries, who had experienced a spontaneous menopause at nineteen years of age. However, the same (47) XXX karyotype has since been noted in some women with established fertility [*6,47,127*], in others with regular menses [*72*] and in a nine year old girl with precocious puberty characterized by menses and breast development since birth [*114*]. A large number of similar cells was found in the (45/47) XO/XXX mosaic of a fifty-two year old woman who had never menstruated [*72*].

The presence of more than two X chromosomes has also been described in phenotypic males with microorchidism (as in Klinefelter's

* It is possible that the (*47*) XXY condition in Klinefelter's syndrome may be an instance of X polysomy in an XY male, in which case it should be included in this group. The finding of a (48) XXXY karyotype in certain phenotypic males with features of Klinefelter's syndrome tends to support this idea. However, it is equally likely that XXY-Klinefelter patients may actually be XX chromosomal females with an extra Y, in which case they cannot be regarded as an example of a poly-X condition. Until this point is settled it is preferable to place these patients in a separate group.

TABLE V

THE KARYOTYPE AND CLINICAL STATE IN INDIVIDUALS WITH X-POLYSOMY*

Karyotype	Sex Chromatin†	Clinical State	Mentality	Reference
		Female Phenotype		
(47) XXX	+ (with 2)	Fertility	Deficient	127;47;6
(47) XXX	+ (with 2)	Regular menses	Deficient	72
(47) XXX	+	Precocious puberty	Not stated	114
(47) XXX	+ (with 2)	Premature menopause	"Average"	68
(47/45) XXX/XO	+ (with 2)	Primary amenorrhea	"Low normal"	72
		Male Phenotype		
(48) XXXY	+ (with 2)	Micro-orchidism as in Klinefelter's syndrome	Deficient	34;10;9;6
(48/49) XXXY/XXXXY	+ (with 2 and 3)	Micro-orchidism	Deficient	70

* See footnote, p. 420.

† (with 2) and (with 2 and 3) indicate that some nuclei contain two and three sex chromatin bodies, respectively.

syndrome) by Ferguson-Smith et al. [*34*] and by Barr et al. [*6,9,10*]. The karyotype found in each of three individuals was (48) XXXY. Varying instances of higher degrees of X polysomy will probably be discovered in view of the findings (1) by Jacobs et al. [*70*] of a (48/49) XXXY/XXXXY mosaic in a man with microorchidism, and (2) by Anders and Prader [*1b*] of 3, 4 and 5 X chromosomes in a mentally retarded, cryptorchid boy with multiple congenital anomalies.

Although the number of cases is relatively small it appears that the hyperdiploid state relative to the X chromosome is closely linked to oligophrenia in both sexes. Phenotypic females with more than two X chromosomes may have normal sexual function but are likely to be mentally defective. On the other hand, it would seem that gonadal as well as mental function is apt to be seriously impaired in phenotypic males with more than one X chromosome.*

Of considerable interest is the observation that the great majority of these phenotypic female and male individuals have an unusual sex chromatin pattern. In addition to a high proportion of chromatin-positive nuclei, almost all show two sex chromatin masses in many of their nuclei. Moreover, certain male patients with more than three X chromosomes have not only one and two sex chromatin bodies in many nuclei but also show a third in some nuclei. The significance of these interesting nuclear sex findings will be discussed.

While mental deficiency seems to be extremely common in polysomic X states, the reverse does not hold true. Employing routine buccal smear examinations in an institution for mentally defective subjects, Ferguson-Smith [*31*] found a little more than 1 per cent of males to be chromatin-positive with evidence of Klinefelter's syndrome (and presumably of a (47) XXY karyotype). In a similar screening procedure of the female patients in the same institution, Fraser et al. [*47*] observed double sex chromatin bodies and a (47) XXX karyotype in four patients, suggesting that the incidence of a polysomic X condition among female oligophrenics is 0.7 per cent.

These anomalous sex chromosome constitutions are most probably due to non-disjunction of daughter X chromosomes. Insufficient data are available to indicate whether or not maternal age plays any role here, as it does in mongolism. A (47) XXX karyotype can result from meiotic

* Barr et al. [*10*] described a mentally defective male (Case 3) with two masses of sex chromatin in many nuclei. He was otherwise normal and the testes showed a nearly normal histologic structure. Although chromosome analysis was not performed, the finding of double sex chromatin bodies strongly suggests the presence of more than two X chromosomes. If this is subsequently demonstrated, it would indicate that X polysomy in phenotypic males is not necessarily accompanied by impaired gonadal function.

TABLE VI
REPORTED KARYOTYPES IN MONGOLISM

Chromosome No.	Type of Chromosome Abnormality	Reference
47	Trisomy for no. 21	86–88;69;15
48	Trisomy for no. 21 plus XXY (with Klinefelter's syndrome)	39;84;85
46	Trisomy for no. 21 and monosomy for no. 16	14
46	Trisomy for no. 21 and monosomy for no. 12	14
46	Reciprocal translocation between no. 21 and no. 15	108;104;20
46	Reciprocal translocation between no. 21 and no. 22	104
46	Reciprocal translocation between two no. 21 *or* trisomy for no. 19 and monosomy for no. 21	45

non-disjunction during oögenesis with the formation of an XX ovum which is then fertilized by a normal X-bearing sperm. (Fig. 9.) An alternate explanation involves mitotic non-disjunction at the first cleavage of a normal XX zygote. In this instance XXX and XO daughter cells are produced, in which case it is necessary to postulate elimination of the XO cell. Production of the (48) XXXY chromosomal constitution requires two or more divisional errors. Meiotic non-disjunction may occur in one or both parents (Fig. 9) either of whom may already be hyperploid for X, or it may take place first during gametogenesis to be followed by mitotic non-disjunction in the first cleavage of an XXY zygote. In this case loss of the daughter XY cell would be presumed to occur. Repeated non-disjunction and simple loss of an X or Y chromosome along a variety of pathways explains the production of polysomic X mosaics. In these instances, persistence of unbalanced daughter cells produces multiple stem-lines of cells.

Mongolism. First reports [*15,69,86,87*] indicated that mongolian idiots have an additional small acrocentric chromosome, causing a modal number of forty-seven. The extra chromosome has been identified most often as one of the two smallest autosomes, usually number 21. The same autosome anomaly has been reported in certain patients who exhibit both mongolism and chromatin-positive Klinefelter's syndrome [*39,84,85*]. Such individuals have forty-eight chromosomes because of their abnormal XXY sex chromosome constitution.

Although the majority of mongols are clearly trisomic for chromosome number 21, with a modal number of forty-seven, more recent studies indicate that some mongols may have a modal number of forty-six. (Table VI.) In such instances an additional autosome aberration accounts for the reduction of the chromosome count from forty-seven to forty-six despite the presence of triplicated number 21 chromosome material. The additional autosome anomaly consists of monosomy or of the centric fusion type of reciprocal translocation. The net effect of either of these divisional errors during gametogenesis is the production of a gamete, and ultimately of a zygote, with one less than the expected number of chromosomes. For example, Böök [*14*] described two mongols with trisomy for chromosome number 21; one patient was monosomic for chromosome number 16 and the other for number 12. In the mongols analyzed by Polani [*108*], Penrose [*104*], Carter [*20*] and their associates there was evidence of reciprocal translocation between chromosome number 15 and one of the three chromosomes number 21. The translocation-produced new chromosome, "15/21," was larger than the original number 15 and stood apart as a single unmatched chromosome corresponding to the pairs of chromosomes in group 6–12. In patient A reported by Penrose et al. [*104*] one of the three chromosomes number 21 appears to be reciprocally translocated with chromosome number 22. The nature of the chromosome aberration in the mongol studied by Fraccaro et al. [*45*] is not fully resolved. It is interpreted as showing either a reciprocal translocation between two of the triplicated number 21 chromosomes or a condition which is trisomic for chromosome number 19 and monosomic for number 21.

Chromosome analysis in the relatives of mongoloid patients has yielded important data pertaining to the origin and transmission of the disease. While trisomy is due to non-disjunction it is impossible without additional information to know whether this divisional error occurred during early embryogenesis of the affected individual or whether it took place during gametogenesis or embryogenesis of one of the parents or even the grandparents. The effect of maternal age in mongolism [*103*] favors the notion that in many instances the defect developed during oögenesis and not during spermato-

genesis. The finding of a normal karyotype in both parents clearly places the error at or after parental gametogenesis.

However, certain studies have revealed the presence of abnormal karyotypes in some parents and relatives of mongols. For example, Fraccaro et al. [*45*] found a chromosome count of forty-seven in the clinically healthy father of a mongol boy. The karyotype interpretation was that the father was trisomic for chromosome number 19. The precise significance of this observation with regard to the cause and transmission of mongolism is not clear. In any event it suggests that trisomy is not necessarily accompanied by phenotypic pathology.*

More extensive studies of familial mongolism by Penrose et al. [*104*] and by Carter et al. [*20*] indicate the existence of phenotypically normal "carriers" of a chromosomal anomaly which is responsible for mongolism in certain offspring. In both of these investigations evidence of a reciprocal translocation involving chromosomes number 21 and 15, with a total count of only forty-five chromosomes, was found in the normal mother and maternal grandmother of mongol patients. Thus in each pedigree there were indications that the responsible abnormality had its origin at least as early as the embryogenesis of the grandmother.

Although these "carriers" have only forty-five chromosomes the latter are balanced for their content of chromosomes number 15 and 21 since they have one each of numbers 15 and 21 as well as the "15/21" translocation product. Hence they are phenotypically normal. However, gametogenesis in such individuals results in ova, half of which are balanced and half unbalanced for chromosome number 21. Balanced ova may contain either a number 15 and 21 or a "15/21" while unbalanced ova may have either a number 15 (without a number 21) or "15/21" and 21. In the latter case it is evident that unbalanced ova bearing both "15/21" and 21 contain almost double quantities of chromosome number 21 although their haploid number is the normal 23. When such ova are fertilized by normal sperm, the resulting zygote has the normal diploid number of forty-six chromosomes and it contains chromosomes number "15/21," 21, 21 and 15. Therefore it is virtually trisomic for number 21 and is equivalent to the condition prevailing in most mongoloids with forty-seven chromosomes.

On the other hand, syngamy between a normal gamete and a balanced *n* minus 1 gamete containing the "15/21" translocation restores the phenotypically normal "carrier" state, i.e., a zygote with forty-five chromosomes which is balanced for chromosomes number 21 and 15 since it possesses numbers 15, 21 and "15/21." Excellent diagrams showing the suggested origin, genetic consequences and mode of inheritance of this type of reciprocal translocation in familial mongolism are presented by Polani et al. [*108*] and by Carter et al. [*20*].

Multiple Congenital Abnormalities. When it became evident that mongolism is due to a chromosome anomaly it was logical to investigate other congenital disorders. Accordingly, karyotype analysis has been performed in a variety of human teratologic conditions involving diverse and multiple systems. These studies have been only partially rewarding in that an apparently normal karyotype has been found in the great majority of individuals with more than forty different kinds of congenital abnormalities and hereditary disorders, exclusive of sexual anomalies. (Table VII.) It should be emphasized, however, that in most instances only one patient with each condition has been examined cytologically.

Chromosomal aberrations have been encountered thus far in a limited number of non-mongoloid patients. (Table VIII.) The latter are mainly infants and young children whose multiple congenital anomalies are usually of a serious nature and seldom conform to a specific clinical entity or syndrome. All are mentally retarded. The karyotypic abnormalities of this relatively small group are characterized by trisomy, translocation or satellite disturbance. This suggests that there are a variety of pathogenetic mechanisms and that many more examples of chromosome abnormalities in multiple congenital malformations await discovery. Reported instances follow.

Autosomal trisomy: Extra autosome in group 16–18: Edwards et al. [*30*] described a chromatin-positive female infant with a heart defect, webbing of the neck, shield-like chest, mental retardation and many minor abnormalities. Normal ovarian tissue was present. The modal chromosome number was forty-seven with an

* A similar karyotype with forty-seven chromosomes due to trisomy for chromosome number 19 was found by Böök [*14*] in a forty-eight year old woman with a congenital auricular septal defect of the heart and no other abnormalities. She had two children, one of whom had the same cardiac lesion.

TABLE VII

LIST OF CONGENITAL ABNORMALITIES AND HEREDITARY CONDITIONS (EXCLUSIVE OF MONGOLISM AND SEXUAL ANOMALIES) SHOWING AN APPARENTLY NORMAL KARYOTYPE (FROM COMPILATIONS BY FORD [*36*] AND HARNDEN, JACOBS AND BUCKTON [*58*])

Achondroplasia
Acrocephalosyndactyly (Apert's disease)
Albright's syndrome
Amaurotic idiocy
Amyotonia
Anencephaly
Arachnodactyly (Marfan's syndrome) *
Arthrogryphosis
Chondrodystrophy
Conjoined twins
Crouzon's disease (dysostosis craniofacialis)
Cyclops deformity
Dwarfism:
Primordial
Hypopituitary
Ehlers-Danlos syndrome
Epiloia
Fröhlich's syndrome
Gargoylism (Hurler's syndrome)
Gaucher's disease
Hemophilia (chromatin-negative "girl")
Huntington's chorea
Hydatidiform mole †
Hydrocephalus
Laurence-Moon-Biedl syndrome
Little's disease
Mental defective:
With hemophilia
With malformed ears; webbed toes; absent fifth metarcarpal
Microcephaly
Muscular dystrophy
Neurofibromatosis
Osteogenesis imperfecta
Phenylketonuria
Sympodia with eventration and renal agenesis

* Abnormal satellites have been found in familial cases (see Table VIII).

† Abnormal numbers of chromosomes have been found in the cells of the villi of hydatidiform moles [*128*].

XX sex chromosome constitution. The additional chromosome, apparently identical with a pair in group 16–18 (Denver system), is believed to be the result of non-disjunction during gametogenesis in one of the parents.

Patau et al. [*102*] mentioned two infants with a similar type of autosomal trisomy and a total count of forty-seven chromosomes. These were a male and an unrelated female with anomalies of the ears, hands and feet, apparent mental retardation, spasticity and congenital heart disease. The extra chromosome appeared to belong in the same group. The fact that the mother of each of these infants was about forty-six years of age at the time of conception suggests that non-disjunction of the implicated autosome had occurred during oögenesis and not in spermatogenesis.

Extra autosome in group 13–15: Patau et al. [*102*] described a chromatin-positive female infant with cerebral defect, apparent anophthalmia, cleft palate, hare lip, simian creases, polydactyly, cardiac defect and mental retardation. The genitalia were normally feminine. The karyotype was abnormal in that it consisted of forty-seven chromosomes, the extra chromosome being identical with one of the pairs in group 13–15. An unrelated female infant with a similar combination of congenital abnormalities and the same abnormal chromosomal complement is also mentioned by these authors.

Extra autosome number 22 (Sturge-Weber syndrome): Hayward and Bower [*60*] described a young boy with the findings ("port-wine" nevus and extensive calcification in the right parieto-occipital region with a gyral distribution) of the Sturge-Weber syndrome together with a small head contour, left hemiparesis and mental retardation. Karyotype analysis revealed a total chromosome count of forty-seven with trisomy of one of the very small acrocentric chromosomes, probably number 22. A normal male type of XY sex chromosome constitution was present.

Extra autosomes numbers 8 and 11: Fraccaro et al. [*46*] described a chromatin-positive boy with cardiac and renal defects, peculiar facies, malformed external genitalia, underdeveloped testis and mental retardation. The chromosome count was forty-nine with apparent trisomy for chromosomes numbers 8 and 11 and a sex chromosome complement of XXY. The authors do not regard this karyotype interpretation as conclusive, particularly because the two trisomics are in group 6–12 in which it is difficult to identify individual chromosomes with certainty. The fact that the patient is chromatin-positive is compatible with the XXY interpretation and the absence of more than one sex chromatin body in any of the cultured nuclei renders it unlikely that more than two X chromosomes are present. However, since the sex chromatin may diminish in number or disappear in cells during culture [*75*], the possibility of multiple sex chromatin bodies, indicative of X polysomy, is not entirely excluded. (The interpretation of this karyotype was subsequently revised. See Addendum under "Poly-X Conditions.)

TABLE VIII

ABNORMAL KARYOTYPES REPORTED IN MULTIPLE CONGENITAL ABNORMALITIES (WITHOUT MONGOLISM OR DOMINATING SEXUAL ANOMALIES)

Sex	Chromosome Abnormability	Karyotype	Clinical Condition	Reference
F	Trisomy in group 16–18	(47) XX	Cardiac defect, webbed neck, shield-like chest, mental retardation	30
F	Trisomy in group 13–15	(47) XX	Apparent anophthalmia, cleft palate, hare lip, simian creases, polydactyly, cardiac defect, cerebral defect, capillary hemangiomas, mental retardation	102
M	Trisomy for no. 22	(47) XY	Sturge-Weber syndrome, mental retardation	60
M	Trisomy in group 6–12 (apparently for no. 8 and 11) and of the sex chromosomes	(49) XXY	Cardiac and renal defects, peculiar facies, malformed external genitalia and testis, mental retardation	46
M	Complete trisomy (triploidy)	(69) XXY	Porencephaly, micrognathia, syndactyly, mental retardation	16
M	Translocation of no. 22 to one of group 13–15	(45) XY	Malformations of vertebral column, small head contour, abnormal sella turcica, mental retardation (polydysspondylism)	135
M	Enlarged satellite on no. 21	(46) XY	Marfan's syndrome (familial)	133
M	Enlarged satellite on no. 18	(46) XY	Marfan's syndrome (familial)	133
F	Enlarged satellite on no. 21	(46) XX	Cleft palate, fused vertebrae, cardiac defect, abnormal ears, short neck	19
F	Elongated satellite on no. 21	(46) XX	Hare lip, rudimentary nose and eyes, cerebal defects, adrenal hypoplasia	19

"Trisomy of a large chromosome": The recent literature contains two other reports claiming to be examples of trisomy involving a large chromosome. De Carli et al. [*26*] describe a "paramongol" woman who appeared to be mongoloid only in the upward slant at the outer corners of her eyes. She was mentally retarded but had no other congenital anomalies. Menstruation was more than adequate. The nuclear sex was chromatin-positive and double sex chromatin masses were present in some of the buccal smear nuclei. The chromosomal number was forty-seven with an extra chromosome of medium size. The latter corresponded to the pairs of chromosomes at the smaller end of the range in group 6–12 and the authors interpreted it to be an *extra chromosome number 10 or 11.*

Sandberg et al. [*113*] described a mentally deficient non-mongoloid, normally menstruating woman with minor congenital abnormalities including webbing of the neck and a flat occiput. Her mother's and father's ages at the time of conception were forty-one and forty-nine years, respectively. Here, too, double sex chromatin bodies were present in some buccal nuclei and the modal chromosome number was forty-seven. The *extra chromosome* in this case was judged to correspond to the *sixth largest* of the set.

The authors of both of these reports considered but rejected the possibility that the extra chromosome in each instance might be an X chromosome. Nevertheless, the grounds for rejection are viewed with skepticism because of the established correlation between multiple sex chromatin structures and mental deficiency on the one hand and X polysomic conditions on the other. Difficulties in the identification of the X chromosome, especially in unusual karyotypes, and the possibility of a small deletion of one of the X chromosomes, as stressed by Jacobs et al. [*71*], render it more likely that these cases are examples of trisomy for the X chromosome rather than for a large autosome.

Triploidy (complete trisomy): Böök and Santesson [*16*] recorded the first instance of polyploidy in man. This was a mentally and physically retarded male infant with porencephaly, micrognathia, syndactyly and mental retardation. The genitalia were normal. The chromosomal count was sixty-nine, indicating the presence of three haploid sets, i.e., three sets of twenty-two autosomes plus three sex chromosomes, XXY. Thus this case is trisomic for all the chromosomes whereas the previous cases are examples of trisomy for a single chromosome. In this patient the data suggest that an ovum

which was non-disjunctive for all the chromosomes (containing 44 plus XX) had been fertilized by a normal Y-bearing sperm (having a 22 plus Y constitution) or that a normal (22 plus X) ovum had united with a totally non-disjunctive sperm (containing 44 plus XY).

Autosomal translocation: Turpin et al. [*135*] reported an abnormal karyotype in a young boy described as having *polydysspondylism* (multiple malformations of the vertebral column, small head contour and bony closure of the sella turcica) with mental retardation. The chromosome count was forty-five apparently the result of translocation (with centric fusion) of chromosome number 22 to one of the chromosomes of group 13–15.

Abnormal satellites: Abnormalities in the size and shape of the satellites of a single chromosome have been described in individuals whose karyotype was otherwise entirely normal. Tjio et al. [*133*] described two unrelated males with familial Marfan's syndrome. A markedly enlarged satellite was present in one instance on one chromosome of pair number 21 and in the other on one of the pair designated by the authors as the eighteenth. Buckton and Harnden [*19*] found enlarged satellites on one chromosome, number 21, in a five year old girl with cleft palate, fused vertebrae, cardiac defect, abnormal ears and a short neck without webbing. In another patient, a newborn female, these workers noted the satellites on one of the chromosomes, number 21, to be elongated. The significance of satellite abnormalities is not clear. However, it is probable that they are not specific in their phenotypic expression since they can arise in a variety of ways involving duplication or translocation [*133*]. The fact that the defect is heterozygous in each described instance is consistent with its presence as a hereditarily transmitted phenomenon.

Leukemia. The presence of an abnormal karyotype in human leukemia was first demonstrated by Ford, Jacobs and Lajtha [*38*]. Most of the marrow cells of this patient with an unspecified type of leukemia contained forty-four chromosomes plus an additional minute fragment. Subsequent studies by Ford and his group [*36*] of a total of ten leukemic individuals disclosed one with a modal count of forty-eight and another with a modal number of forty-six including a minute fragment.

Chromosome aberrations, consisting of abnormalities in number or form or both, were found by Baikie et al. [*3,4*] in five of six patients with acute leukemia but in none of eight with chronic myeloid or lymphatic leukemia or in two with myelomatosis. Accordingly, these investigators introduced the interesting possibility of a fundamental difference between acute and chronic forms of leukemia. They deem it likely that some forms of acute leukemia are the result of changes in the genetic material of the cell.

Because of the possibility that tissue culture may enable normal marrow cells to outgrow aneuploid neoplastic cells with more exacting nutritional requirements, Sandberg et al. [*114*] fixed the marrow cells immediately after procurement from the patient. With avoidance of tissue culture and pretreatment with colchicine, these investigators found numerical and/or structural abnormalities in three of five patients with acute leukemia and in each of two patients with chronic leukemia. An abnormal karyotype was also noted in uncultured marrow cells of one of three patients with acute leukemia by Bottura et al. [*18*]. Most of the cells contained fifty-three chromosomes.

For the present, the significance of the abnormal karyotypes reported thus far in leukemia is unclear. It is not yet known whether the chromosomal aberrations are cause or effect of the disease. Since all studies showing chromosomal anomalies employed marrow cells, and because of the technical difficulty in distinguishing counts of normal from those of leukemic cells, it is possible that the observed abnormalities simply represent the non-specific variations encountered in neoplastic cells. The use of skin cultures in addition to marrow studies might clarify this point. In this manner it should be possible to learn whether other cells of the body contain normal complements of chromosomes when the marrow cells show gross chromosomal abnormalities. The finding by Johnston [*77*] in skin cultures of four patients with acute leukemia and two with chronic leukemia of a high proportion of hyperploid cells, despite the normal modal number, suggests that chromosome changes in leukemia may be reflected outside the hematopoietic system.

X-Ray Treatment and Chromosome Damage. The results of serial studies of chromosome changes in blood cultures of patients subjected to x-ray therapy have been reported recently by Tough et al. [*134*]. Two patients with ankylosing spondylitis were treated. One received a total skin-

TABLE IX

SUMMARY OF CHROMOSOME FINDINGS REPORTED IN VARIOUS CLINICAL STATES*

Clinical State	Sex Chromatin	Chromosome Constitution
Normal male	−	(46) XY
Normal female	+	(46) XX
Pseudohermaphroditism		
Male	−	(46) XY
Female	+	(46) XX
True hermaphroditism	+	(46) XX
	−	(46) XY
Klinefelter's syndrome	+	(47) XXY, (47/46) XXY/XX, (48) XXYY
	−	(46) XY, (47/46) XXY/XY
Turner's syndrome	−	(45) XO, (45/46) XO/XX
	+	(45) XO, (45/46) XO/XX, (46) XX
"Pure" gonadal dysgenesis	−	(46) XY
"Atypical" gonadal dysgenesis	±†	(46) Xx
	−	(45) XO
Poly-X conditions		
Female phenotype		
Fertility or premature menopause	+	(47) XXX
Primary amenorrhea	+	(47/45) XXX/XO
Male phenotype		
Microorchidism	+	(48) XXXY, (48/49) XXXY/XXXXY
Mongolism		(47) with trisomy for no. 21
		(46) with trisomy and monosomy or translocation
Multiple congenital anomalies‡		(47) with trisomy for various autosomes
		(49) XXY with triple trisomy
		(69) XXY, complete trisomy
		(45) with centric fusion
		Abnormal satelites
Leukemia‡		Abnormal numbers and forms
Radiation effects		Abnormal forms and numbers

* For details and references see text and individual tables.
† Sex chromatin bodies (small) in 7 per cent of buccal smear nuclei.
‡ Chromosome abnormalities in some but not all instances.

dose of 1,500 rads in ten equal fractions over the spinal column and sacroiliac regions. The other was given a single dose of 250 rads over the spine alone and was studied by serial examinations over a period of ten days. The data indicated that large, accumulated x-ray dosage results in considerable chromosome damage characterized by significant changes in the count distribution and various structural abnormalities such as chromosome fragments, ring chromosomes and chromosomes with two centromeres (dicentrics). In the patients treated with a single dose, there was a reduction in the percentage of cells with forty-six chromosomes, a corresponding increase in the proportion with forty-seven, and an increase in cells with structural abnormalities. These changes began to recede significantly by the fifth day.

The importance of these preliminary observations lies not only in their revelation of the type of chromosome damage resulting from radiation therapy but in the prospects for establishing relationships between radiation dose and extent of chromosome damage in man.

A summarizing compilation for the entire group is presented in Table IX.

CORRELATION WITH SEX CHROMATIN

Nuclear sex, as determined by the sex chromatin pattern, is a well established parameter in the evaluation of clinical conditions in which the true sex is uncertain. In most mammals, including man, the majority of resting nuclei of normal females contain the characteristic sex chromatin structure of Barr [*5,8*]. These nuclei are designated "chromatin-positive." Chromocenters of identical size, shape and position are rarely

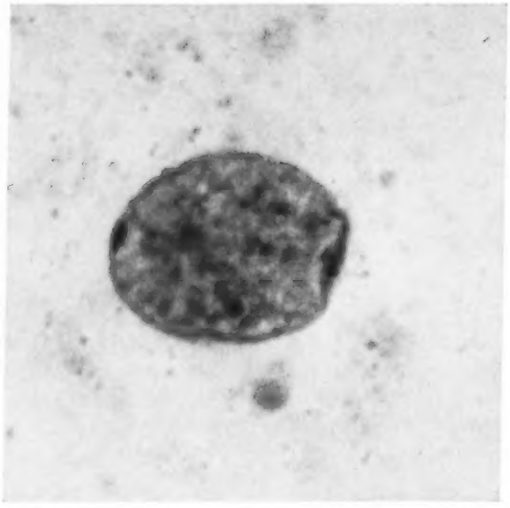


FIG. 10. Buccal smear nucleus of female showing the characteristic planoconvex sex chromatin body in contact with the nuclear membrane. Smaller, non-specific chromocenters are visible within the nucleoplasm. Unfixed preparation stained with methylene blue, magnification × 2,500.

if ever found in the nuclei of normal males, who are accordingly termed "chromatin-negative."

The sex chromatin body of female nuclei may assume various shapes but it is usually planoconvex, one side being flattened against the nuclear membrane. (Fig. 10.) It is recognizable in the human and macaque embryo only after the first week of gestation [*100*] but it persists thereafter throughout the life of the individual. Its presence is unaffected by spontaneous or induced alterations in the sex hormonal status of the host [*122*] and it can be identified as well in neoplastic tissues of females [*121*]. Its mean dimensions are 0.8 by 1.1 μ with minor differences in size from one cell to another [*7*]. Recent studies indicate that its dimensions may be significantly reduced by extraneous factors such as pharmacologic agents [*120*].

In contrast with the laborious aspects of chromosome analysis, the sex chromatin test provides a relatively simple method by which it is possible in *normal* individuals to determine whether resting nuclei have one or two X chromosomes. This is tantamount to knowing whether the sex chromosome complement is XY or XX. The same close correlation between the nuclear sex chromatin pattern and the chromosomal sex has been found in several types of anomalous sexual development, notably in male and female pseudohermaphroditism and in most instances of true hermaphroditism.

However, two important developments have revealed the existence of serious limitations to the accuracy of the sex chromatin test in the detection of chromosomal sex. These appear precisely where such information is needed most, i.e., in *abnormal* individuals. First, the discovery of discordances between nuclear and phenotypic sex (chromatin-positive patients with Klinefelter's syndrome, chromatin-negative patients with Turner's syndrome and patients with the syndrome of testicular feminization) and, second, the demonstration by chromosome analysis that chromatin-negative individuals are not always XY and that chromatin-positive persons are not invariably XX. For example, the sex chromosome constitution of chromatin-negative patients with Turner's syndrome is usually XO [*40,44,73*] and is occasionally an XO/XX mosaic [*41,114*] but not XY. (Table IV.) Moreover, an XO complement may also be found in chromatin-positive patients with Turner's syndrome [*32,55*] and an XXY constitution is frequent in chromatin-positive patients with Klinefelter's syndrome [*39,74,97*].

Interestingly enough, the usefulness of the sex chromatin test in the differential diagnosis in sex anomalies is considerably enhanced by some of these very discrepancies. As an example, most patients with Turner's syndrome and all of those with the syndrome of testicular feminization are chromatin-negative. The absence of sex chromatin in the nuclei of these phenotypic females may be an important aid in establishing the diagnosis. Also, approximately half of the individuals with Klinefelter's syndrome are chromatin-positive and this finding in these phenotypic males is of considerable diagnostic help. Male and female pseudohermaphroditism can be distinguished from one another by their respective sex chromatin patterns.

In certain abnormal conditions resting nuclei may contain more than one mass of sex chromatin. These nuclei are in addition to those with single sex chromatin bodies. Such individuals are usually of subnormal intelligence and, with one exception [*24*], have more than two X chromosomes. (Table V.) Double sex chromatin particles have been found in phenotypic females with a (47) XXX karyotype [*6,47,72,127*] and in one who was a 45/47 XO/XXX mosaic [*72*]. They have also been noted in phenotypic males with microorchidism like that in Klinefelter's syndrome, three with a (48) XXXY constitution [*6,9,10,34*] and one who was a (48/49) XXXY/

XXXXY mosaic [*70*]. In fact, some of the nuclei of the latter patient contained three distinct sex chromatin structures as well.

It thus appears that the finding of multiple sex chromatin bodies in either phenotypic sex strongly suggests the presence of more than two X chromosomes. It would also seem that the number of sex chromatin particles is usually one less than the number of X chromosomes present. That this is not invariably true is evident from the finding of a (45) XO karyotype in a chromatin-positive patient with Turner's syndrome [*32,55*].

Origin of the Sex Chromatin. It was originally suggested [*5,8,50*] that the appearance of the sex chromatin depends upon the presence of the XX chromosome pair. Barr [*7*] has summarized the very strong if not conclusive evidence that the sex chromatin is derived from heterochromatic chromosome regions that display positive heteropycnosis in resting nuclei. The early hypothesis that it develops from positively heteropycnotic portions of two X chromosomes in somatic association is supported by direct cytologic evidence. The studies of Reitalu [*111*], Klinger [*79,80*] and James [*75,76*] leave little doubt that the sex chromatin in normal man and higher mammals is composed of two closely applied but separate portions, each of which is consistent with a chromosome segment.

Attempts have been made to investigate this problem by non-cytologic genetic technics, such as color vision testing, in individuals with anomalous sexual development. Red-green color blindness (daltonism) is determined by a mutant gene believed to be situated in the non-homologous portion of an X chromosome. Since the gene is recessive its presence in a male results in color blindness because its action is unopposed. In females, the condition occurs only when the gene is present in *both* X chromosomes. The incidence of red-green color blindness (in the British Isles) is 7 to 8 per cent of normal males and 0.6 per cent of normal females [*106*]. Thus the condition is approximately twelve to thirteen times as frequent in males as it is in females. Accordingly, when an individual is found to have defective red-green color vision it is statistically much more likely that one rather than two X chromosomes are present.

The results of color vision testing in chromatin-negative patients with abnormal sexual differentiation are consistent with those expected in males. For example, four of thirty-eight individuals with Klinefelter's syndrome [*107*] and four of twenty-nine with gonadal dysgenesis [*11*] were found to be color-blind. Colorblindness also occurs not infrequently in the syndrome of testicular feminization [*67,90,124*]. Conversely, in a group of chromatin-positive patients, none of eleven with gonadal dysgenesis or Ullrich's syndrome [*11*] and none of fifty-five with Klinefelter's syndrome [*107*] exhibited color blindness. While these findings suggest that the sex chromatin occurs in the presence of two X chromosomes and fails to appear when only one is present, a much larger series is needed. This is indicated by a more recent study in which three of thirty-four chromatin-positive individuals with Klinefelter's syndrome were found unexpectedly to be color-blind [*98*]. These observations disagree sharply with those of the aforementioned investigation of a similar group of patients. They are explained by the authors as being due to, possibly, the loss by one X chromosome of a small euchromatic fragment bearing the dominant allele for normal color vision. In this manner the recessive mutant gene on the other X chromosome results in color blindness whereas preservation of heterochromatic portions of both X chromosomes produces the sex chromatin.

It now appears that certain data derived from simultaneous studies of the sex chromatin and chromosome constitution make it difficult to accept the concept that the sex chromatin always originates from portions of two X chromosomes. Current notions in this respect may require revision in the light of the following observations.

(1) The finding of double sex chromatin bodies in the nuclei of patients with three X chromosomes (see Table v) and the observation of triple sex chromatin masses in a (48/49) XXXY/XXXXY mosaic individual [*70*] and in a boy with multiple congenital anomalies whose nuclei contained three, four or five X chromosomes [*1b*]. If the sex chromatin arises from portions of two X chromosomes, the presence of double sex chromatin structures should mean that four X chromosomes, not three, are present in these nuclei. Similarly, triple bodies should indicate the presence of six X chromosomes.

(2) The demonstration of an XO sex chromosome complex in a chromatin-positive patient with Turner's syndrome. Here it is obvious that the sex chromatin body could not have been derived from two X chromosomes since only one was present. These data in man suggest as an

alternative explanation the possibility that the sex chromatin may be produced by heterochromatic portions of a single X chromosome. Certain observations in some lower species appear to support this concept.

(3) Ohno et al. [*99*] described positive heteropycnosis in only one of the two X chromosomes in squash preparations of prophase nuclei of mouse ovarian follicular cells and of rat regenerating liver cells. The single positively heteropycnotic X chromosome was believed to produce the sex chromatin mass of the interphase nucleus, its sharply bent shape accounting for the occasionally observed bipartite appearance of the sex chromatin.

(4) The sex chromatin is found in the nuclei of females, the heterogametic sex, in certain insects [*118*] and in domestic fowl [*83*]. The sex chromosome complex of these females is XO. Sex chromatin is absent in the nuclei of the male homogametic (XX) sex of these species.

The foregoing considerations raise serious doubts that the sex chromatin is always formed from two X chromosomes. While the data obtained from lower species have not yet been confirmed, the cited observations in man leave little doubt that sex chromatin may be derived from a single X chromosome, at least under certain abnormal conditions.* Further studies in normal and abnormal individuals at the chemical and metabolic levels of the cell itself are needed to resolve the question as to whether the findings in chromosomally abnormal persons and in lower animal forms justify conclusions in normal man.

* Stewart [*125*] suggests that the sex chromatin body may be derived from only one of the two X chromosomes, even in normal persons. This explanation is based on the assumption that the heterochromatin of only one X chromosome is metabolically active during interphase and is therefore not stainable. The heterochromatin of any other X chromosomes is thus free to be stainable since it is functionally inert at this time. Mention should be made of alternative hypotheses regarding the origin of the sex chromatin. Segal and Nelson [*117*] proposed the idea that the sex chromatin may be derived from the heterochromatic material of a pair of autosomes carrying male-determining factors. An entirely different and unique concept has been advanced by Iverson [*66*] who explains the observed differences in male and female nuclear morphology as due to a difference on a molecular level in their respective template-replica systems. In this case the X chromosomes would not be the sole precursors of the sex chromatin and nuclear sex dimorphism would then be representative of secondary sexual characteristics at the level of the nucleus. Somewhat along the same line of thought are the opinions of Hienz [*62*] and Hertl [*61*] who believe that morphologic differences between the nuclei of both sexes are the result of a sex-linked difference in nuclear metabolism.

SUMMARY

An attempt is made to review critically the newer work dealing with human chromosome analysis and to correlate it with nuclear sex chromatin studies. A definition and explanation of cytogenetic terms in the form of a glossary is included.

Recent technical advances, especially the use of short-term cell culture and pretreatment with colchicine, have made possible a reasonably detailed and accurate study of the morphologic characteristics of individual human chromosomes and of their total number per nucleus. Although numerous procedural modifications and systems for the designation of chromosomes have been introduced by various investigators, there is now at least universal agreement on an international standard of nomenclature (Denver system). Currently, the principal sources of cells employed for analysis are those of bone marrow obtained by aspiration, skin cells provided by a tiny biopsy, and circulating leukocytes procured by simple venipuncture. The application of chromosome analysis simultaneously to multiple sources of tissue cells yields more authentic karyotype interpretations than those derived from a single type of tissue cell. Accordingly, karyotype analysis of more than one type of tissue cell is preferable, and virtually mandatory when dealing with unusual clinical conditions or with atypical chromosome constitutions.

The composition of the normal human karyotype and the manner in which it is obtained from study of metaphase nuclei is discussed in detail. Various pitfalls and potential sources of error in the sorting and matching of chromosomes are described. A full understanding of them as well as an awareness of certain limitations still inherent in present technics is essential if misleading interpretations are to be avoided.

Because they play a decisive role in the evolution of most of the reported types of chromosome abnormalities, some of the more evident cytogenetic mechanisms are discussed. Deviations from the normal structure of chromosomes are usually due to translocation or partial deletion. Abnormal chromosomal counts are the result of non-disjunction, simple loss, mosaicism or centric fusion.

The karyotypes reported in various morbid states are presented. The interpretation of many

of the abnormal ones must be regarded as presumptive rather than as definitive. In the majority of instances there is no close correlation between karyotypes and disease patterns. It is stressed that the finding of a normal chromosome constitution, with currently available technics, does not exclude the possibility that a chromosome aberration may exist nevertheless.

The sexual anomalies show a surprising lack of close correlation between chromosome constitutions and clinical types. In male and female pseudohermaphroditism, the karyotypes are those of normal men and women, respectively. True hermaphrodites examined thus far usually exhibit a normal chromosome count with a sex chromosome constitution which appears to be concordant with the nuclear sex chromatin pattern. Klinefelter's syndrome and Turner's syndrome may be characterized by karyotypes which are different from the "preferred" patterns of (47) XXY and (45) XO, respectively.

Three or more X chromosomes (X polysomy) may be present in the nuclei of phenotypic females and males. Gonadal function may or may not be impaired in such individuals but mental deficiency is a very frequent finding.

In the majority of instances, mongolism is due to triplication of the chromosomal material of autosome number 21. The total count may be forty-seven or forty-six. In the latter instance the absence of an abnormal total number is accounted for by the occurrence of trisomy for chromosome number 21 in association with reciprocal translocation or monosomy. Studies of the relatives of mongoloid patients indicate that the disease may be transmitted by phenotypically normal "carriers" of a chromosomal anomaly.

A number of different chromosome aberrations have been found in patients with a variety of congenital abnormalities, seldom conforming to a specific clinical entity or syndrome. These are principally infants and young children. Mental retardation was present in each instance. It is now evident that aneuploidy in various forms, involving autosomes or sex chromosomes, is a concomitant, if not an etiologic, factor in certain forms of mental deficiency.

Abnormalities in the number and structure of chromosomes have been demonstrated in patients with leukemia and in those exposed to radiation therapy. Available data are too meager and fragmentary to warrant meaningful conclusions.

Wherever possible, the nuclear sex chromatin pattern in reported cases has been correlated with the chromosomal constitution. This has led to the discovery that certain chromatin-positive individuals with sexual anomalies may have only one X chromosome and that persons with double sex chromatin masses in their nuclei may have three X chromosomes. The obvious inference is that the sex chromatin, contrary to previously held hypotheses, is not always formed from two X chromosomes. The problem of the origin of the sex chromatin is discussed, including the possibility of its derivation under certain circumstances from positively heteropycnotic regions of a single X chromosome.

The accumulated data in patients with sexual anomalies indicate that (1) chromatin-negative individuals may have an XY or XO sex chromosome constitution, (2) chromatin-positive patients may be XO, XX or XXY, (3) double sex chromatin masses usually indicate the presence of three X chromosomes, and (4) the finding of *small* sex chromatin bodies is consistent with but not necessarily indicative of partial deletion of one X chromosome.

ADDENDUM

As could be anticipated, many additional observations became available or were reported since this manuscript was prepared. Primarily, these consist of previously undescribed examples of poly-X conditions, sex mosaicism, translocations and autosomal trisomy. Some reports deal with further studies of the normal karyotype, others with newly discovered chromosome aberrations in various clinical states. A few concern revised interpretations of previously evaluated karyotypes. The grouping of the added data conforms to that employed in the manuscript.

The Normal Karyotype. That the currently established normal number of chromosomes (forty-six) is not an effect of *in vitro* culture, colchicine treatment or fixation was shown by the demonstration of identical karyotypes in uncultured, non-treated, unstained bone marrow mitoses [*184*].

Patau [*183b*] has stated that much of the numbering of chromosomes in published reports is haphazard and lacking in specificity. He attributed this to the claim that the Denver system of classification, because of technical limitations, provides biologically meaningful definitions for only ten of the twenty-three pairs of chromosomes (groups 1–3, 4–5, 16–18, 19–20). In order

to avoid inaccuracies of interpretation, especially in the "6–12 and X," 13–15 and 21–22 groups, he prefers (1) the usage of group symbols (A through G) for the seven chromosome groups of the Denver system, and (2) the assignment of numbers to individual chromosomes *only* when their identification is reasonably certain. The periodical in which the majority of published human karyotypes has appeared, *The Lancet* [*138b*], conceded the validity of Patau's discussion but urged continued adherence to the now well established Denver system until a more authorative alternative emerges.

Supernumerary chromosomes, as described by Kodani [*81,82*] in testicular germ cells, could not be found by Makino and Sasaki [*176*] in any of the somatic nuclei of fifty-four different Japanese embryos.

The matter of *satellited chromosomes* continues to receive special consideration. Drawing attention to the findings of Tjio et al. [*133*] of enlarged satellites in familial Marfan's disease, McKusick [*174*] pointed out the presence of giant satellites in individuals unafflicted by this disorder. Moreover, this investigator questioned whether the two cases described by Tjio et al. were really valid examples of Marfan's disease. Thus, there is some doubt as to whether this type of satellite abnormality is the specific underlying factor.

More recently, Ferguson-Smith and Handmaker [*157*] presented evidence that five (not three according to the Denver system) pairs of chromosomes in man are satellited. These are the acrocentric chromosomes number 13, 14, 15, 21 and 22. If this is the normal situation, according to these investigators and to Patau [*183b*], the presence or absence of satellites can no longer be used as a criterion in the identification of a chromosome pair. In this event, the autosome trisomy in mongolism may not necessarily be restricted specifically to number 21, as is currently held. Moreover, there is a possibility that the size of satellites on any particular chromosome may be characteristic of the individual's karyotype and not necessarily related to congenital disease.

Ferguson-Smith and Handmaker [*157*] also noted that satellited chromosomes appeared to be attached to one another by their satellites. This phenomenon of "*satellite association*" was observed so often as to suggest that it may be one of the factors involved in the production of certain human chromosomal aberrations, i.e., autosomal non-disjunction and reciprocal translocation.

A delightfully readable account of the essential facts dealing with human karyotypes, chromosome abnormalities and clinical manifestations has been presented as "Chromosomes for Beginners" by Lennox [*173b*].

The available information on the chromosomal cytology and karyotypes of the order Primates has been reviewed by Chu and Bender [*150*].

True Hermaphroditism. A normal female karyotype, (46) XX, was found in three chromatin-positive individuals by Miller [*177*].

Male Pseudohermaphroditism. A normal male karyotype, (46) XY, was demonstrated in three patients by Lejeune et al. [*173a*].

Klinefelter's Syndrome. A second chromatin-positive patient with Klinefelter's syndrome and mental retardation having a (48) XXYY karyotype (Table III) has been described by Carr et al. [*147b*].

A chromatin-positive subject studied by Lejeune et al. [*172*] showed two types of chromosome anomaly. In addition to an XXY sex chromosome complex produced by non-disjunction, there was evidence of a centric fusion type of reciprocal translocation between a chromosome number 14 and a 15. The latter aberration is responsible for the observed modal number of forty-six instead of the expected forty-seven.

In a chromatin-positive individual who also had chronic myeloid leukemia, Tough et al. [*193*] found a (46/47) XY/XXY mosaic constitution in nuclei cultured from peripheral blood (culture of the skin yielded only a (47) XXY complex). A similar mosaic had been described previously only in a chromatin-negative patient [*114*]. The "Philadelphia chromosome" (see under Leukemia) was also noted in the present patient.

Turner's Syndrome. In the nuclei obtained by bone marrow aspiration of a chromatin-*negative* patient, Sandberg et al. [*186*] found a (46) XX karyotype. Previously (Table IV), this karyotype was known only in a chromatin-positive individual.

"*Male*" *Turner's Syndrome.* Although Turner's syndrome exists exclusively in phenotypic females, many of the characteristic somatic congenital anomalies have been described in phenotypic males. When these are associated with testicular dysgenesis, the affected individual

may be regarded as an instance of the male counterpart of Turner's syndrome.

The chromosomal constitution has been determined in eight such chromatin-negative individuals [*138a,151a,159–161,183a,188*]. In each instance a normal male karyotype was demonstrated. In a chromatin-positive phenotypic male with testicular germinal aplasia and many of the congenital anomalies encountered in Turner's syndrome, Oikawa and Blizzard [*183a*] found a (46) XX chromosomal constitution. Analysis of the karyotype revealed the presence of one normal X chromosome and an "enlarged" X chromosome corresponding in size to those of pair number 3. Parenthetically, this case is another of the rare instances in which testicular development may occur in the apparent absence of a Y chromosome.

Abnormal X Chromosome. A normal number of chromosomes was found by Edwards [*155*] in a chromatin-positive woman of normal physique and good intelligence with primary amenorrhea. However, karyotype analysis revealed the absence of one chromosome, presumably an X, and the presence of an abnormal chromosome, resembling most closely a number 9 to which was added an extra segment. The author believes the most likely interpretation to be a translocation of a long arm of an X chromosome on to the long arm of an autosome of similar proportions (number 9) or possibly on to the long arm of another X.

In the blood cell cultured nuclei of a chromatin-positive fourteen and a half year old girl with stunted growth and poor sexual development, Blank et al. [*142*] encountered a (45/46) XO/XX mosaic. Analysis of the (46) XX stemline cells revealed an extra chromosome corresponding to pair number 3 and one less than is normal in group 6–12+X. The authors' interpretation is that the cells with forty-six chromosomes contain one normal X and an isochromosome for the long arm of the other X. The karyotype findings and interpretation are similar to those described earlier by Fraccaro et al. [*43*] in certain patients with gonadal dysgenesis.

A similar X chromosome aberration in a chromatin-positive individual with Turner's syndrome and a (46) XX chromosome constitution (see Table IV) was described by Ferguson-Smith [*156*]. In this instance there was one normal X chromosome and one abnormal chromosome comparable in proportions to number 3. The anomaly was interpreted as being the result of a translocation or isochromosome formation involving the other X.

The same type of karyotype abnormality was found by Oikawa and Blizzard [*183a*] in a chromatin-positive phenotypic male with several of the congenital anomalies of Turner's syndrome. A modal number of forty-six chromosomes was present with one normal X and an "enlarged" X chromosome, resembling those of the third pair. Incidentally, this case is an example of testes developing in the apparent absence of a Y chromosome.

An identical karyotype was encountered by Carr et al. [*149*] in a chromatin-positive infant with clinical features suggestive of Turner's syndrome. Here, too, there were forty-six chromosomes with only fifteen chromosomes in the 6–12 group, one of which was interpreted to be an X chromosome. In addition there appeared to be an extra chromosome, corresponding to those of pair number 3, which was interpreted as an isochromosome of the long arms of X. However, since normal ovaries were found subsequently at autopsy, it was deemed impossible to be certain whether the isochromosome represented the long arms of X or of an autosome of similar proportions, such as number 6. In the latter event, of course, both X chromosomes would be normal.

"Pure" Gonadal Dysgenesis. Netter et al. [*180*] reported a (46) XY karyotype in a chromatin-negative phenotypic female with primary amenorrhea due to histologically verified gonadal dysgenesis. Stature was normal and there were no congenital defects suggestive of Turner's syndrome.

Poly-X Conditions, Sex Mosaicism. Two more examples of a (48) XXXY constitution have been reported by Carr et al. [*148*] in Klinefelter-type individuals. Double sex chromatin bodies were present in the buccal smear nuclei as well as in the nuclei of the skin and Leydig cells.

A new type of X polysomy, (49) XXXXY, was reported in chromatin-positive males by Fraccaro and Lindsten [*158*] and by Miller et al. [*178*]. The first of these reports concerns a re-interpretation of a previously published karyotype [*46*]. A chromatin-positive boy with forty-nine chromosomes was thought to have an XXY sex chromosome complex and extra autosomes number 8 and 11 (Tables VIII and IX). In view of the subsequent demonstration of buccal smear nuclei containing two, three and even four Barr bodies, suggesting the presence

of X polysomy, the two extra chromosomes in the 6–12 group were now interpreted as additional X chromosomes rather than as autosomes. The individual with multiple sex chromatin bodies studied by Miller et al. [*178*] also showed a presumptive (49) XXXXY chromosome constitution.

Another new addition to the group of poly-X conditions are the two unrelated females with a (48) XXXX karyotype reported by Carr et al. [*147a*]. Triple Barr bodies were present in the buccal smear nuclei of these mentally defective women who were otherwise normal.

An XO/XY mosaic in a fifty-five year old chromatin-negative woman with primary amenorrhea and mental retardation was studied by Blank et al. [*141*]. At the age of twenty-two years a laparotomy revealed a small left ovary, a very small uterus and no gonad on the right side.

An XO/XX mosaic in a fourteen and a half year old chromatin-positive girl was demonstrated by Blank et al. [*142*]. Growth was stunted and sexual maturation was inapparent. There were no congenital anomalies suggestive of Turner's syndrome and the urinary excretion of gonadotropin was normal.

A woman with high intelligence was found by Carr et al. [*146*] to be an XO/XXX sex mosaic. These observations represent an exception to the rule that most poly-X individuals are mentally retarded.

An XY/XXY mosaic constitution was encountered in a fetus by Klinger et al. [*169*]. Of great interest is the fact that sex chromatin mosaicism was also demonstrated. Some areas of the amnion were chromatin-negative while others were chromatin-positive. Apparently, the degree of mosaicism (sex chromosome and sex chromatin) can vary considerably from one region of the body to another. These findings re-emphasize the fact that mosaicism can easily escape detection when nuclei from but one region or source are studied.

A completely new type of sex chromosome mosaicism, XO/XYY, has been studied by Cooper et al. [*152*] in a phenotypic female with many features of Turner's syndrome and "streak ovaries." Karyotype analysis of cells grown from a skin biopsy specimen revealed a (45) XO chromosome constitution. Examination of cultured peripheral blood leukocytes indicated a modal chromosome number of forty-seven with an XYY sex chromosome pattern. This report is the first to describe (1) the presence of two Y chromosomes in human nuclei with only one X chromosome and (2) the absence of testicular tissue in a person possessing the Y chromosome. It is also of interest that this is apparently an instance of system mosaicism rather than tissue mosaicism.

Mongolism. A new observation is that of Hanhart et al. [*163*] who found forty-seven chromosomes, apparently of the "standard type," in a mongol mother and her mongol daughter.

Jérôme et al. [*166*] noted a disturbance of tryptophan metabolism in a group of mongolian idiots who were trisomic for chromosome number 21. These individuals had a low urinary excretion of 5-HIAA which suggests the possibility of a low serotonin content in the brain. Thus, it may be possible to relate chromosomal changes to biochemical alterations [*171*].

A new karyotype, exhibiting "21-trisomy/normal mosaicism," has been described by Clarke et al. [*151b*] in a young girl with certain features of mongolism but no evident physical or mental retardation. To the authors it seems possible that mosaic mongols (sic) may sometimes be phenotypically normal.

Multiple Congenital Anomalies—Autosome Trisomy. Anders and Prader [*1b*] studied a mentally retarded boy with bilateral cryptochidism and numerous congenital abnormalities whose buccal smear nuclei contained double and triple Barr bodies. The cultured marrow nuclei contained 3, 4 and 5 additional chromosomes in the number 6–8 range. These were regarded as X chromosomes in view of the sex chromatin findings. In addition, many nuclei contained one or two additional chromosomes of the size range 15–17. (This case is referred to in the manuscript under Poly-X Conditions.)

Five patients with a variety of anomalies were described by Smith et al. [*187*]. Each had a new type of autosomal trisomy with a modal chromosome number of forty-seven and an extra chromosome in the E group of Patau (16–18 Denver) probably number 18. A similar karyotype in a seriously malformed child of a hyperthyroid mother was described by van Wijck et al. [*195*].

Following the observation by Hayward and Bower [*60*] of trisomy for autosome number 22 in the marrow nuclei of an individual with the Sturge-Weber syndrome, fifteen additional patients with this disease have been subjected to karyotype analysis [*153,162,164,170,196*]. In each

instance a normal chromosome constitution was found. It is, therefore, not unlikely that the finding in the original case, subsequently confirmed in the peripheral blood [*164*], was merely coincidental.

Autosome Translocations. A translocation between chromosomes number 13 and 22, identical with that found earlier in polydysspondylism [*135*], was reported by Moorhead et al. [*179*] in four children with speech and mental retardation. Because this is an instance of the centric fusion type of reciprocal translocation, with loss of the minute fragment causing a reduction in the chromosome number by one, the total chromosome count was forty-five. The identification of the same karyotype in the normal mother indicates that the translocation heterozygosity was familial and that it need not necessarily be associated with phenotypic abnormalities.

A previously undescribed example of the centric fusion type of reciprocal translocation involves chromosomes number 14 and 15. Lejeune et al. [*172*] found a (46) XXY karyotype with a "14/15" translocation-product in a chromatin-positive individual with Klinefelter's syndrome.

Another new variety of reciprocal translocation, between autosomes number 2 and one of group 6–12 (probably number 10), was identified by Böök et al. [*143*] in cultured nuclei of the skin of a normal woman. An almost identical aberration, involving autosomes number 2 and "probably number 9," was found by Trujillo et al. [*193*] in the cultured leukocytes of an infant with osteogenesis imperfecta congenita. Of considerable importance is the conclusion of the investigators that the abnormal chromosomes in their case had arisen *in vitro*.

With the accumulation of more and more data, it appears that (1) the presence of satellites may play a role in the translocation mechanism, (2) the chromosomes involved are always or nearly always acrocentric and (3) the presence of translocations seems to increase the probability of non-disjunction in other chromosomes during gametogenesis [*145,194*]. Moreover, it has been suggested by Böök et al. [*143*] that translocation heterozygotes might not be insignificant in human populations and that they may constitute an unrecognized factor in fetal mortality and/or in the production of viable but defective offspring.

Leukemia. A minute acrocentric chromosome in the cultured circulating leukocytes of patients with chronic myeloid leukemia was first described by Nowell and Hungerford [*181*]. The modal number of chromosomes was forty-six. Shortly afterward, Baikie et al. [*139*] confirmed this observation and interpreted the unusually small chromosome as a partially deleted autosome number 21 or 22. The abnormal chromosome could not be identified in skin cultures nor was it present in earlier studies of marrow cells of some of these individuals. In an extended study by the same group, Tough et al. [*192*] designated the abnormal chromosome as Ph^1 to indicate the geographical location, Philadelphia, of the laboratory in which the chromosome was first noted by Nowell and Hungerford. The Edinburgh group found the Ph^1 chromosome in twelve of fifteen patients with chronic myeloid leukemia and in one of three others who had passed into a phase of acute leukemia. It was not encountered in any of five cases of acute leukemia associated with mongolism. It is their conclusion that the cells with the Ph^1 chromosome are leukemia cells and that the abnormal chromosome is due to deletion of material from the long arm of one of the chromosomes number 21 or 22 or to translocation. It thus appears that the cell containing the Ph^1 chromosome is in effect a specifically labelled cancer cell and that the Ph^1 chromosome is the first tumor cell marker to be discovered [*154*].

The significance of the relationship between leukemia and mongolism is provocative and unresolved. The finding that the same autosome, number 21, may be involved in both chronic myeloid leukemia and mongolism suggests a chromosomal substrate for the fact that mongoloid subjects have an increased susceptibility to leukemia. However, the leukemia of mongolism is acute leukemia and none of the subjects with mongolism and acute leukemia studied by Tough et al. [*192*] and by Johnston [*167*] showed the Ph^1 abnormality.

Radiation Effects. Employing blood cell cultures, Boyd et al. [*144*] observed that the administration of ablative doses (100 mc.) of radioiodine to patients with thyroid adenocarcinoma results in a decrease in the proportion of cells with a normal chromosomal number and in the appearance of cells with structural abnormalities, i.e., dicentric chromosomes (with two centromeres) and fragments. These changes are similar to those described by Tough et al. [*134*] following a single x-ray dose of 250 rads applied to the spine in the treatment of ankylosing spon-

dylitis. Following the use of smaller doses of I^{131}, of the order of 10 mc., in the treatment of hyperthyroidism, there was some suggestion that recognizable chromosomal changes (increase in abnormal mitoses) may occur.

A similar type of chromosomal aberration was found by Stewart and Sanderson [*190*] in a chromatin-positive patient with Klinefelter's syndrome shortly after exposure to diagnostic radiation. The observed abnormality was an abnormally long and apparently dicentric chromosome. Although this might have been induced by diagnostic radiation, the possibilities remain that it might have been related to the patient's abnormal (47) XXY karyotype or that it could have arisen in culture.

Malignant Tumors. In a systematic analysis of the chromosomal cytology of a variety of human tumors, Makino et al. [*175*] and Tonomura [*191*] found the number of chromosomes of the tumor cells to show a pronounced variation within a wide range. In addition, there was always in each tumor a stem-line (or lines) of tumor cells showing a characteristic abnormal modal number, usually hyperdiploid, and a specific idiogram distinguishable from that of other tumors as well as from that of normal tissue.

The extent of the chromosomal variations encountered in cultures of tumors is probably in excess of the situation as it exists *in vivo*. Puck [*185*] has shown that while normal human cells cultured *in vitro* retain the constancy of their chromosomal number and structure for long periods, hyperdiploid aneuploid cells continually throw off chromosomal variants. This phenomenon is believed to be the result of an unusually great tendency for non-disjunction to occur or for the formation of multiple mitoses.

Derivation of the Sex Chromatin. From studies of human fetuses at about the fourth month of gestation, Ohno and Makino [*182*] showed that the sex chromatin body seen in female interphase nuclei represents a single X chromosome. This finding is consistent with earlier studies in the mouse and rat in which the single X nature of the sex chromatin was first described [*99*].

Further evidence supporting the hypothesis that the sex chromatin is derived from the heterochromatic segment of a single X chromosome is afforded by the report of Stewart and Sanderson [*189*]. These investigators noted the presence of a structure, apparently identical with the sex chromatin body, in about 10 per cent of the germ cell nuclei of normal human testicular tissue. It was most prominent in primary spermatocyte nuclei during very early prophase. If these observations are correctly interpreted, they may account for the reported occurrence of the Barr body in some male somatic nuclei.

A different approach to the nature of the Barr-like body occasionally seen in male somatic nuclei is made by Klinger and Schwarzacher [*168*]. These investigators determined histophotometrically the DNA content of the sex chromatin or heterochromatic bodies in the nuclei of human amnion epithelium and liver parenchyma. Their findings indicate that all male nuclei which have a heterochromatic body possessing the morphologic appearance and DNA content of the sex chromatin of the diploid female nucleus are polyploid. The DNA content of the heterochromatic body of male diploid nuclei is about half that of a sex chromatin body of female diploid nuclei.

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Chromosomal Aberrations in Human Disease*

A Review of the Status of Cytogenetics in Medicine

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SINCE the beginning of 1959 a number of discoveries have led to appreciation of a formerly unknown mechanism in the causation of human disease. These discoveries have demonstrated that some types of human disease may have as their basic defect an abnormality in the number or morphology of the chromosomes. The rapid progress that has been made in this direction has resulted from the application to human material of new technics that have recently become available in the field of cytogenetics.

Cytogenetics may be defined as the direct study of the chromosomes, the bearers of the genetic material, by means of cytologic technics. It differs in its approach from transmission genetics, in which information is obtained by statistical analysis of characteristics transmitted from one generation to the next. In cytogenetics, information is obtained by direct observation of the chromosomes and other cellular elements in terms of their behavior and appearance in cytologic preparations. Correlations may then be made with the phenotypic characteristics of the organism and genetic information thereby obtained.

The methods of cytogenetics applied to lower organisms, such as the fruit fly (Drosophila species) and corn (Zea mais), have provided much important information and corroboration in conjunction with transmission genetics [*1,2*]. In contrast, human cytogenetics until recently has been a relatively unexplored field, contributing little information relating specifically to human problems. It is the purpose of this paper to review some of the recent developments in this field which bear directly on an understanding of certain specific human disorders.

EARLY DEVELOPMENTS

Prior to 1952, the scope of human cytogenetics was limited to attempts to count accurately the number of human chromosomes, and to verify that in the human species, as in other mammalian species, there was a sexual dichotomy in the chromosomes. The male was found to have an X and a Y chromosome, the female had two X chromosomes. This dichotomy was considered to be basic in sex differentiation [*1,2*].

Hansemann [*3*] in 1891 first succeeded in counting the chromosomes in cells of human testicular tissue, and in three cells arrived at the numbers 18, 24 and 40. This work was performed before the realization by Sutton [*4*] and Boveri [*5*] in 1903 that the chromosomes actually contained the hypothesized hereditary units, the genes. In 1912 DeWiniwarter [*6*], also working with histologic sections of testicular tissue, arrived at a human diploid number of forty-seven, claiming that the human male had twenty-three autosomal paired chromosomes and an X chromosome, but no Y chromosome, while the female had twenty-four pairs. In 1921 Painter [*7*], in his preparations of fresh testicular tissue, noted chromosome numbers ranging from forty-five to forty-eight. He also noted the existence of the Y chromosome, a small unpaired chromosome visible during meiosis. It is interesting, in the light of recent work, to quote Painter directly: "In my own material the counts range from forty-five to forty-eight apparent chromosomes although in the clearest equatorial plates so far studied only forty-six chromosomes have been found."

Two years later, in 1923, Painter [*8*] unhesitatingly reported a diploid number of

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forty-eight chromosomes—twenty-three autosomal pairs and two unmatched chromosomes—in normal testicular tissue. This seemed to clear away all doubt as to the human chromosome number, and the count of forty-eight was apparently verified by several other investigators [*9,10*]. As will be pointed out later, the technics employed at that time were inadequate for much further progress in the direct study of human chromosomes, and the situation remained essentially unchanged until 1952.

THE SEX CHROMATIN

While progress in the direct examination of human chromosomes was slow, a significant development occurred in another direction which had important effects on the study of the heredity of sex. In 1950 Barr et al. [*11*] observed a difference in the morphology of cell nuclei, depending on the sex of the individual providing the tissue. This consisted of a small, darkly-staining body located at varying positions within the nucleus, depending on the type of tissue examined. In human skin and buccal mucosa it is recognizable as a distinct chromatin body, usually closely adherent to the inner surface of the nuclear membrane. (Fig. 1.) It is found almost exclusively in the normal female (therefore said to be "chromatin positive") in up to 80 per cent of cells of a section of skin 5 μ in thickness, and rarely if ever in the normal male (therefore designated "chromatin negative") [*12*].

This sexual dimorphism is apparently present in many human tissues, including blood cells. Close study of well prepared peripheral blood films reveals the presence of accessory nuclear lobules or "drumsticks" in normal female polymorphonuclear leukocytes [*13*]. (Fig. 2.) They are observable in low frequencies (average: 3 per cent) and some skill is required to differentiate them from other nuclear irregularities.

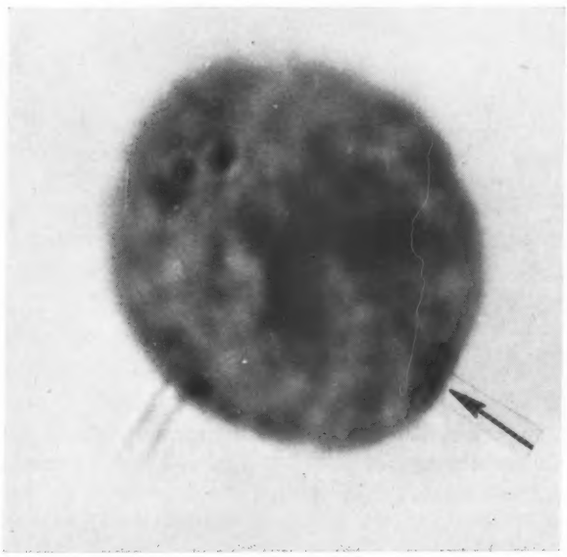


FIG. 1. Sex chromatin body (arrow) in nucleus of buccal mucosal cell from normal female.

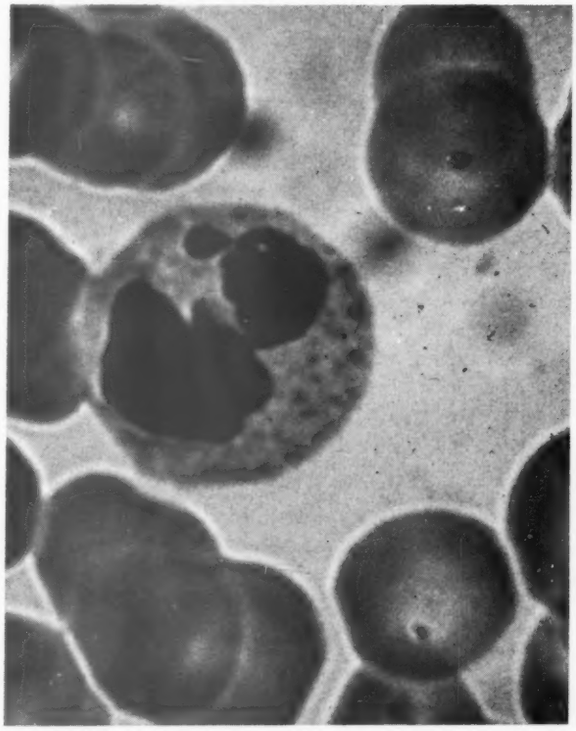


FIG. 2. "Drumstick" in polymorphonuclear leukocyte in peripheral blood smear from normal female.

The exact nature of the sex chromatin body is still under investigation, and the reader is referred to several excellent discussions of the subject [*12,14–16*]. Most recent work suggests that the sex chromatin body represents the heterochromatic portion of one or both X chromosomes. It had been widely believed that the presence of two X chromosomes was necessary for and always associated with the presence of the sex chromatin body. Recent work has generally confirmed this idea, but the finding by Grumbach et al. [*17*] of two patients with chromatin-positive Turner's syndrome, each with only one X chromosome, has cast doubt upon the view that two X chromosomes are required for the sex chromatin body.*

* At least one of these cases demonstrates mosaicism (see Mosaicism, p. 461), with some cells having 2 X chromosomes (Grumbach M., personal communication).

Application of the technic of "nuclear sexing," as it was called, yielded some interesting results when applied to certain cases of human infertility and abnormal sexual development [*18*]. In Klinefelter's syndrome [*19*], usually characterized by male appearance associated with sterility, testicular atrophy, hyalinization of the seminiferous tubules and gynecomastia, a chromatin-positive pattern is found in approximately 80 per cent of cases [*12*]. These individuals may appear to be perfectly normal males although they may have some degree of gynecomastia. Conversely, in Turner's syndrome (or gonadal dysgenesis) the patient appears as a sexually infantile female whose ovaries are represented only by some streaks of connective tissue attached to the broad ligaments. However, the patient is unmistakably female. In these cases, some 80 per cent will have a chromatin-negative pattern, despite the female appearance [*12*]. While these syndromes are interesting endocrinologically, they are even more fascinating from the genetic point of view and will be dealt with in detail subsequently.

TECHNICAL DEVELOPMENTS

Before discussing the recent strides in human cytogenetics, some explanation of the technical progress in this field is necessary. Until the work of DeWiniwarter, most studies of human chromosome morphology were carried out on bits of testicular tissue obtained from executed criminals. Invariably, some time elapsed before the tissue was secured and fixed. This delay undoubtedly resulted in postmortem tissue changes and in large part explains the conflicting results obtained by Hansemann, Bardeleben, Flemming, Duesberg and others as discussed by Painter [*8*]. DeWiniwarter [*6*] realized the importance of careful handling of the tissue, and obtained fresh surgical specimens. He prepared the tissue with meticulous care and sectioned it in thicknesses of 5 to 7.5 μ. His drawings indicate a high degree of clarity, but nevertheless crowding and overlapping, together with the possibility of sectioning through a chromosome bent upon itself, made determination of the chromosome number uncertain. Painter used essentially similar technics, employing surgically obtained testicular tissue, fixed, imbedded in paraffin, stained and sectioned, hence subject to the same errors.

The use of testicular tissue seemed logical to these workers because it was the site of frequent meiotic divisions, and might be expected to provide large numbers of cells for study. However, as already indicated, the rapid degenerative changes that take place in excised testicular material and the necessity for histologic preparation requiring fixation, dehydration, clearing, imbedding in paraffin, sectioning and staining all provide ample opportunity for alterations in the specimen that might lead to faulty interpretation. In addition to these difficulties, the human meiotic chromosomes seen in such preparations are poorly outlined, closely clumped and overlapping, and extremely difficult to count accurately.

The recent improvements in technic are due mainly to the introduction of the methods of tissue culture into this field. Kemp [*20*] in 1929 was probably the first to apply tissue culture methods to the examination of human chromosomes. However, his technics did not permit very clear visualization of the chromosomes. In 1952 Hsu [*21*] found that the addition of colchicine to cultures of human cells grown *in vitro* provided large numbers of cells in the metaphase of mitosis, due to the unique action of colchicine in arresting dividing cells at the metaphase. This was coupled with the use of a hypotonic salt solution during preparation of the cells. The hypotonic solution had the effect of swelling the cells and apparently disrupting the mitotic spindles, thereby greatly spreading the chromosomes and making them more amenable to study. The chromosomes examined by this method present themselves as sharply outlined X-shaped bodies, each representing a single chromosome in the process of dividing (i.e., a pair of chromatids). (Fig. 3.) There is a minimum of overlapping in good preparations and the chromosomes can be counted fairly easily, especially by means of photomicrography.

Using this technic Hsu confirmed Painter's finding of forty-eight chromosomes as the normal diploid number in man. However, examination of the published photographs of Hsu's preparations might lead one to a different result, and in later work Hsu reports forty-six chromosomes, as do most other workers at present [*22*]. Nevertheless, the figure of forty-eight remained unquestioned until 1956, when Tjio and Levan [*23*], working with cultures of fetal lung tissue, reported only forty-six chromosomes at mitosis. This finding was soon confirmed by Ford and Hammerton [*24*] and by other workers, including Chu and Giles [*25*] and Tjio and Puck [*26*]. At the present time, the normal human diploid chromosome number seems clearly established as forty-six. The possibility of the existence of supernumerary chromosomes, a phenomenon known in lower organisms, has been raised by Kodani [*27*] who describes the presence of small unmatched chromosomes in several of his preparations of testicular tissue. However, his findings have not been confirmed by others.

The use of tissue culture methods introduces new difficulties, but these are gradually being overcome. One important problem is the fact that cells in long-term tissue culture tend to change their chromosome number after many serial passages. The cells may become polyploid, translocations may occur or the chromosomes may fragment, increasing the number of

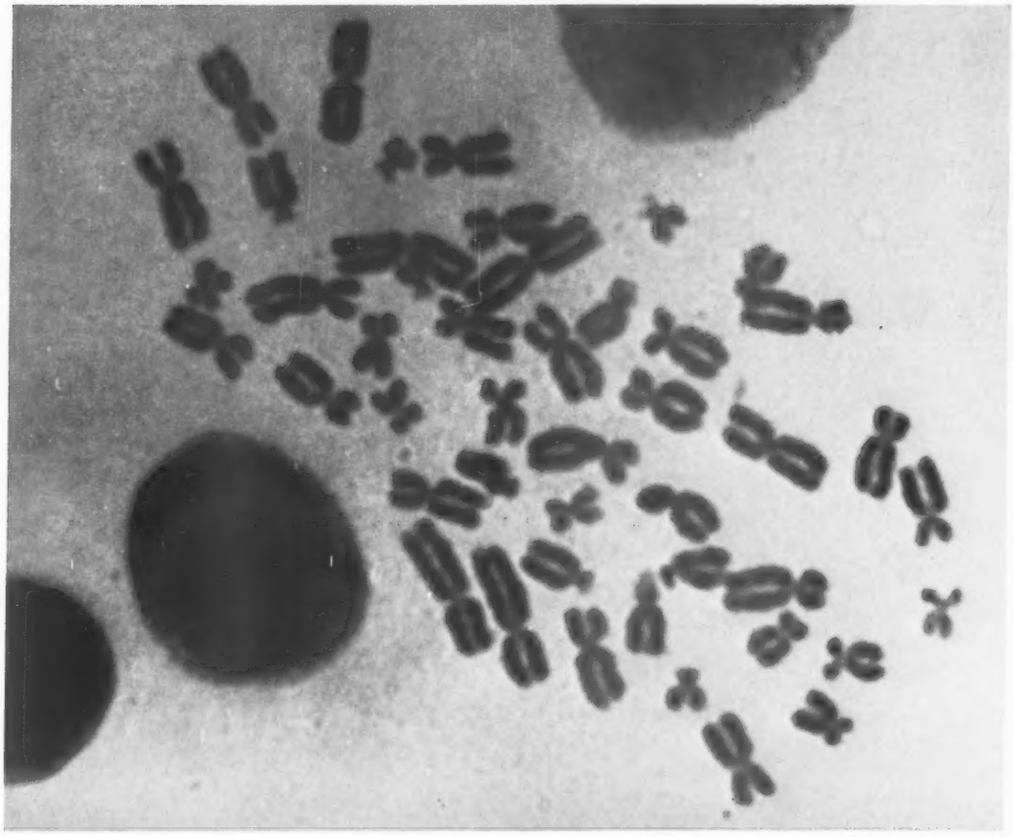


FIG. 3. Human mitotic chromosomes at metaphase. Prepared from peripheral blood grown in tissue culture (see Appendix for method). Forty-six chromosomes.

apparent chromosomes. In one line of originally normal human synovial tissue in our laboratory, after more than one year of repeated serial passages using technics in use prior to those of the past two years, the chromosome number had become exceedingly variable, ranging from fifty-two to over sixty. The alteration may be a result of fragmentation of the chromosomes at the centromere. (Fig. 4.)

In practice, two methods are now available to circumvent such alterations of chromosome number. First, very short-term tissue cultures are used, thus preventing the cumulative effect of long-term cultures with serial passages. Freshly drawn bone marrow is incubated for several hours, followed by another period of incubation in the presence of colchicine. The cells are exposed to hypotonic saline solution, centrifuged, stained and spread on slides for direct examination. This is essentially the method of Ford, Jacobs and Lajtha [*28*] and, with modifications, is used by many workers.

Other innovations which appear to inhibit alteration of chromosome numbers, as demonstrated by Puck [*29*], are the use of a growth medium which is optimal for the cells, rigid control of temperature and pH of the medium with an atmosphere of 5 per cent carbon dioxide, and periodic regrowth of the entire culture from single isolated cells. These modifications seem to allow long-term growth of human tissues without significant chromosomal alterations. At any time after establishment of the cells in tissue culture, they may be grown directly on cover slips, incubated in the presence of colchicine, swelled with hypotonic saline solution, stained and examined. For more exact details as to procedure, the reader is referred to the papers cited.*

The use of colchicine has been avoided by several workers, including Lejeune et al. [*30*], Fraccaro and his associates [*31*], and ourselves. It is the belief of these investigators that to study the morphology of the chromosomes accurately it is essential to introduce the least possible amount of distortion into the preparations. It is known that, in addition to halting the process of mitosis at metaphase, colchicine has the effect of contracting and widening the chromosomes, making their finer morphology difficult to observe. To obviate the necessity of using colchicine, these workers use a technic involving growth of the tissues for an intermediate length of time, long enough to permit growth of a single cell layer on a glass cover

* At present, the majority of workers use the peripheral blood culture method because of its simplicity and reproductivity (see Appendix).

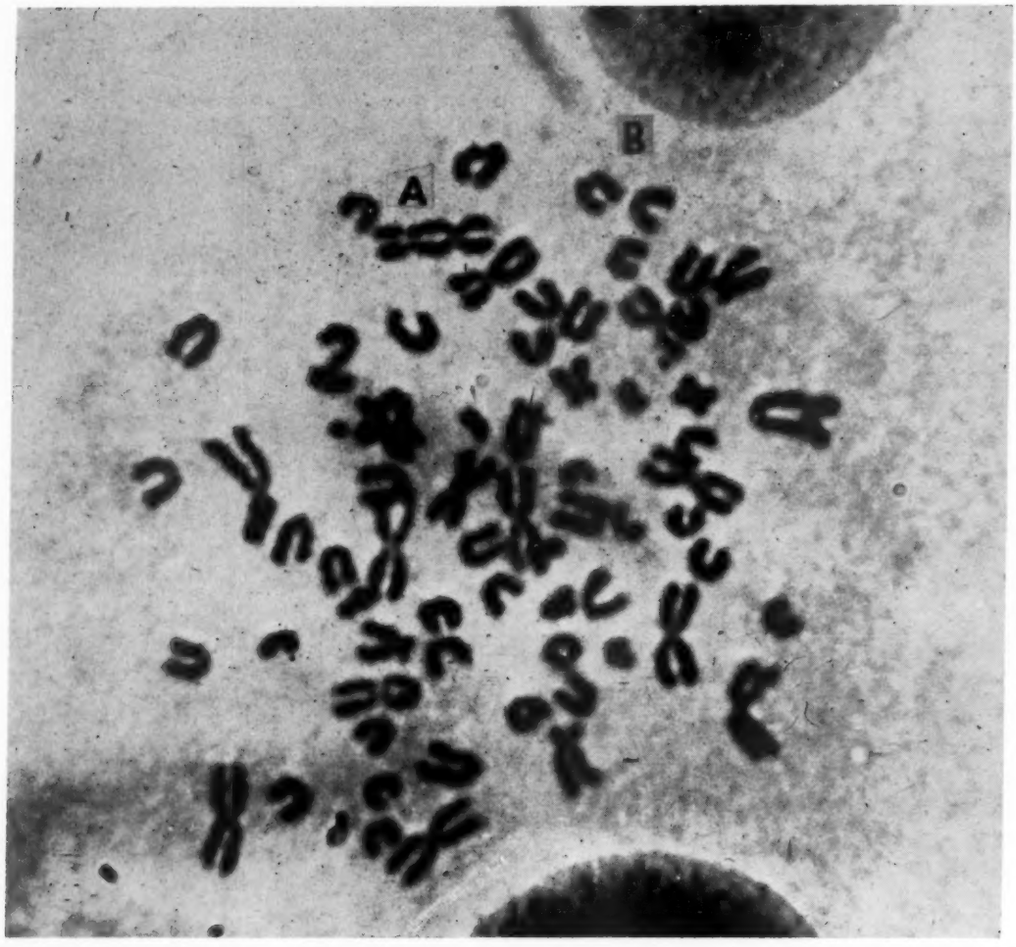


FIG. 4. Chromosomes at metaphase in originally normal human synovial tissue. Sixty-nine chromosomes. Cells grown in culture with multiple passages over a period of two years. Note abnormal chromosome number and morphology. One large chromosome with secondary constriction is seen at (A). Several chromosomes appear to have terminal centromeres (B).

slip. The tissue culture medium is refreshed twelve to sixteen hours prior to examination of the cells, which has the effect of producing a growth spurt, providing many mitotic figures suitable for examination.

The advantages of using colchicine lie primarily in the larger number of mitoses available for examination, and in the greater uniformity of the chromosomes in different cells and different preparations. This uniformity is of importance when careful measurement of the characteristics of individual chromosomes is undertaken.

In the Appendix are presented the methods currently in use in our laboratory for the examination of chromosomes in cells cultured from bone marrow, skin and peripheral blood.

NORMAL CHROMOSOMES—THE HUMAN KARYOTYPE

The use of the technics just described has enabled us to prepare clear, easily examined preparations of human chromosomes (Fig. 3), and to characterize the normal human chromosome complement in some detail. As in any new, rapidly advancing field, differences of terminology among the different workers have developed. The main source of difficulty has been the use of different systems of numbering the chromosomes. At a conference held in Denver, Colorado, in April 1960 [*32*] it was generally agreed to use a numbering system similar to that first proposed by Chu and Giles [*25*], with some modifications. That numbering system will be followed in this paper.

In good preparations the photographs of the chromosomes may be enlarged and the individual chromosomes cut out and paired to provide a matched set, arranged and numbered in order of decreasing size. (Fig. 5 and 6.) Such an arrangement is called an idiogram or karyotype.

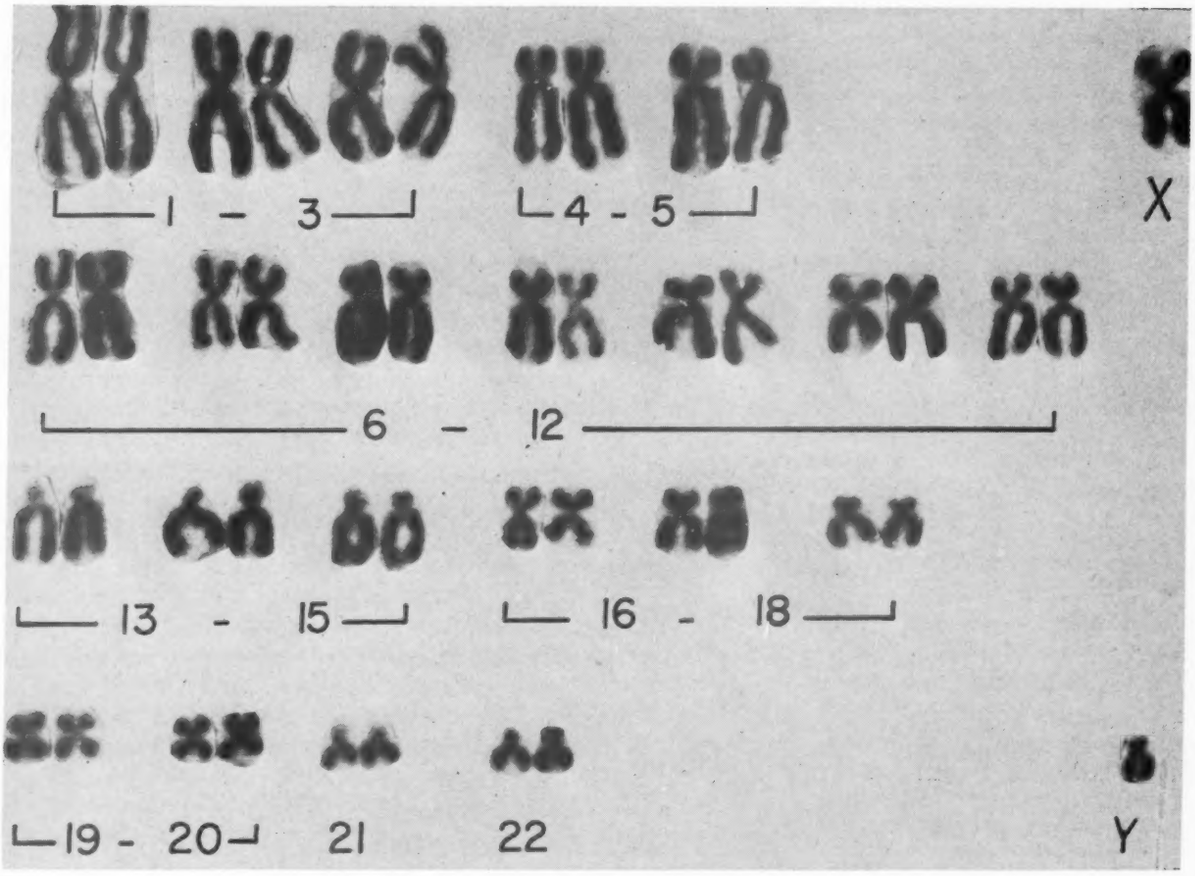


FIG. 5. Karyotype of normal male mitotic chromosomes. Numbered according to the Denver system. Note unmatched X and Y chromosomes.

Each chromosome is characterized by the presence of a centromere, a constriction located somewhere along the length of the chromosome at which the two chromatids that constitute the chromosome in the process of division are closely joined. The location of the centromere, the over-all length of the chromosome, and the length of the "arms" on either side of the centromere are the basic distinguishing features of each chromosome, and permit identification and matching. (Fig. 7.) Three types of placement of the centromere are recognized: (1) median, submedian or metacentric, when the centromere is at or fairly close to the midpoint of the chromosome; (2) subterminal or acrocentric, when the centromere is near but not at the tip of the chromosome; (3) terminal or telocentric, when the centromere is at the tip of the chromosome and no short arm projects beyond it. In the normal human chromosome set there are no truly telocentric chromosomes, although some of the acrocentrics have extremely small "short arms."

In the normal human male (Fig. 5) five pairs of the longest chromosomes can be distinguished, gradually decreasing in size. They all have median or submedian centromeres and can usually be further subdivided into two groups, the first with three pairs and the second with two pairs. These are followed by another seven pairs of metacentrics, smaller and very similar to each other. In fact, the similarity of these fourteen chromosomes is so great that there is much doubt whether they should be paired on a morphological basis [*102*]. Together with these twelve pairs, a single unmatched metacentric chromosome is found which falls midway between the two groups in size, and actually cannot always be distinguished from the members of the sixth or seventh pairs. In the female, however, there is no unmatched chromosome, but instead another matched pair making thirteen pairs of metacentric chromosomes of large to moderate size. (Fig. 6.) By inference the unmatched chromosome in the male has been labelled the X chromosome and the pair of corresponding size

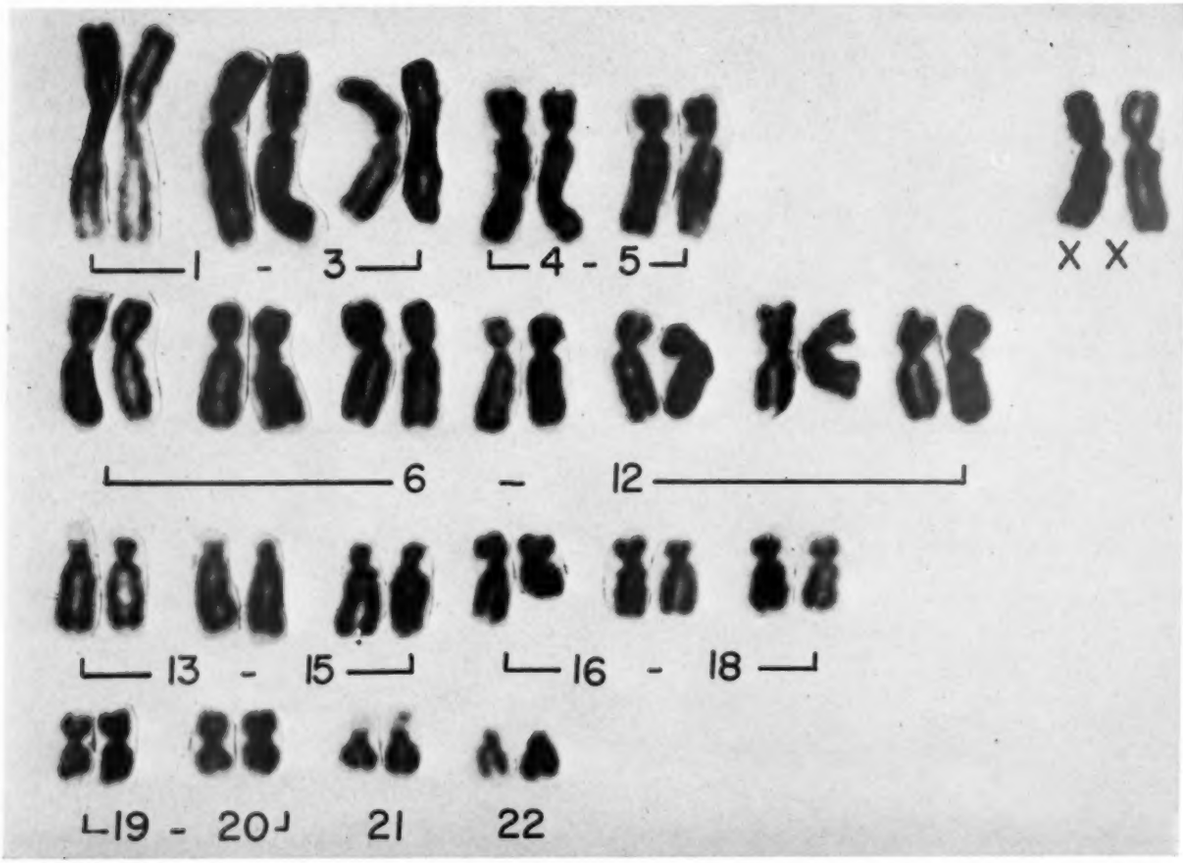
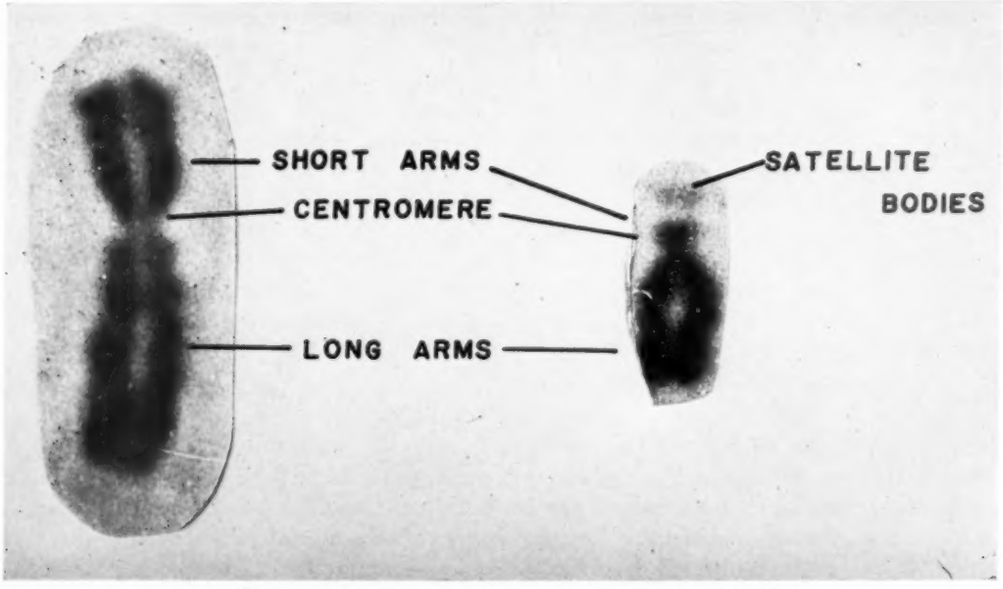


FIG. 6. Karyotype of normal female mitotic chromosomes. Numbered according to the Denver system. Note matching pair of X chromosomes.



7A 7B

FIG. 7. Identifying features of human mitotic chromosomes. A, submedian or metacentric. Note location of centromere, long and short arms, over-all length. B, subterminal or acrocentric. Note location of centromere, extremely small short arms, over-all length; also satellite bodies.

in the female is considered to be the pair of X chromosomes distinguishing the female sex. Identification of the X chromosome is always uncertain, and some investigators claim it cannot be identified at all [*102*].

In addition to these twenty-five or twenty-six chromosomes, we can identify three pairs of smaller acrocentric chromosomes, quite similar in appearance. Two of these pairs can sometimes be distinguished from the third by the presence (in the best preparations) of two small "satellite bodies" extending from their short arms. (Fig. 7.) These three pairs are followed by five pairs of small metacentric chromosomes of decreasing size, the last two being much smaller than the first three. Following these, we find two pairs of tiny acrocentrics. The larger of these pairs is distinguished by satellite bodies similar to those mentioned. Finally, in the male, a single small acrocentric chromosome, without satellite bodies, remains unmatched.* Together with the previously described unmatched metacentric, it completes the twenty-third "pair" of the males making forty-six chromosomes in all. The normal female never possesses this extra small chromosome, and it is presumed to be the Y chromosome of the XY male sex-determining pair.

In addition to the features just described, there appears to be considerable variation in the length of the chromosomes observed, depending on whether the cell is found in early or late metaphase. In early metaphase the chromosomes are long and filamentous, with a knobby appearance. The two chromatids of each chromosome are not widely separated, and often one cannot discern the division between them at all. As metaphase progresses the chromosomes appear to contract and become broader, whereas the chromatids spread more widely apart, their only point of conjunction being the centromere. Finally, they may rarely be seen to have severed their connection even at the centromere, just preparatory to entering anaphase. These differences are not always observed in colchicine preparations because of its effect of stopping mitosis at more or less the same point in all cells.

HUMAN CHROMOSOMAL ABNORMALITIES

Possessed now of a technic for adequately examining human chromosomes, workers in this field turned their attention quite naturally toward application of their methods to human disease. In 1959 Lejeune et al. [*33*] and Jacobs et al. [*34*] described a consistently abnormal chromosome number in the somatic cells of "mongoloid" idiots. These patients were discovered to have forty-seven rather than forty-six chromosomes at mitosis. By preparing a karyotype it is seen that these individuals have three rather than two of the small acrocentric chromosomes with satellite bodies. (Fig. 8.) This is thought by most to be an extra twenty-first chromosome, although in some numbering systems it has been considered to be the twenty-second. Nevertheless, the presence of a whole extra chromosome can be demonstrated consistently, and it is believed that this is the basic defect in the disease.

The possibility that mongolism might be due to a chromosomal abnormality had long been suspected. In the 1930's Waardenburg [*35*], Bleyer [*36*] and Penrose [*37*] each independently suggested that an abnormal chromosome number might explain the facts then known about mongolism. The reasons for postulating a chromosomal abnormality were, basically:

(1) There is 100 per cent concordance between monozygous (identical) twins, both of the pair always being affected, never one without the other. In dizygous (fraternal) twins the reverse is usually the case, only one twin being affected [*38*]. This information essentially rules out the possibility of an intrauterine cause or of any other environmental agent affecting the developing embryo, and suggests a genetic defect.

(2) In any family it is very unusual for more than one child to be affected, and the parents are always normal. Exceptional cases of two or more mongoloid children in one family have been reported, but these are exceedingly rare [*39*]. This mode of occurrence would suggest that the genetic factor for mongolism is inherited as a rare recessive trait, or that it is a fresh mutation in each case. The possibility of fresh mutation is rendered unlikely by the frequency of occurrence of the condition, which is about one per 700 live births [*40*]. No gene is known in man that has such a high mutation rate. The possibility that the condition is due to a dominant gene with much reduced penetrance is also small, since the occurrence of mongolism is almost always a single event in any given family tree [*39*].

(3) On rare occasions mongol females have

* Recent work [*106,131*] has shown that all the acrocentric chromosomes except the Y carry satellites, although they are not always visible.

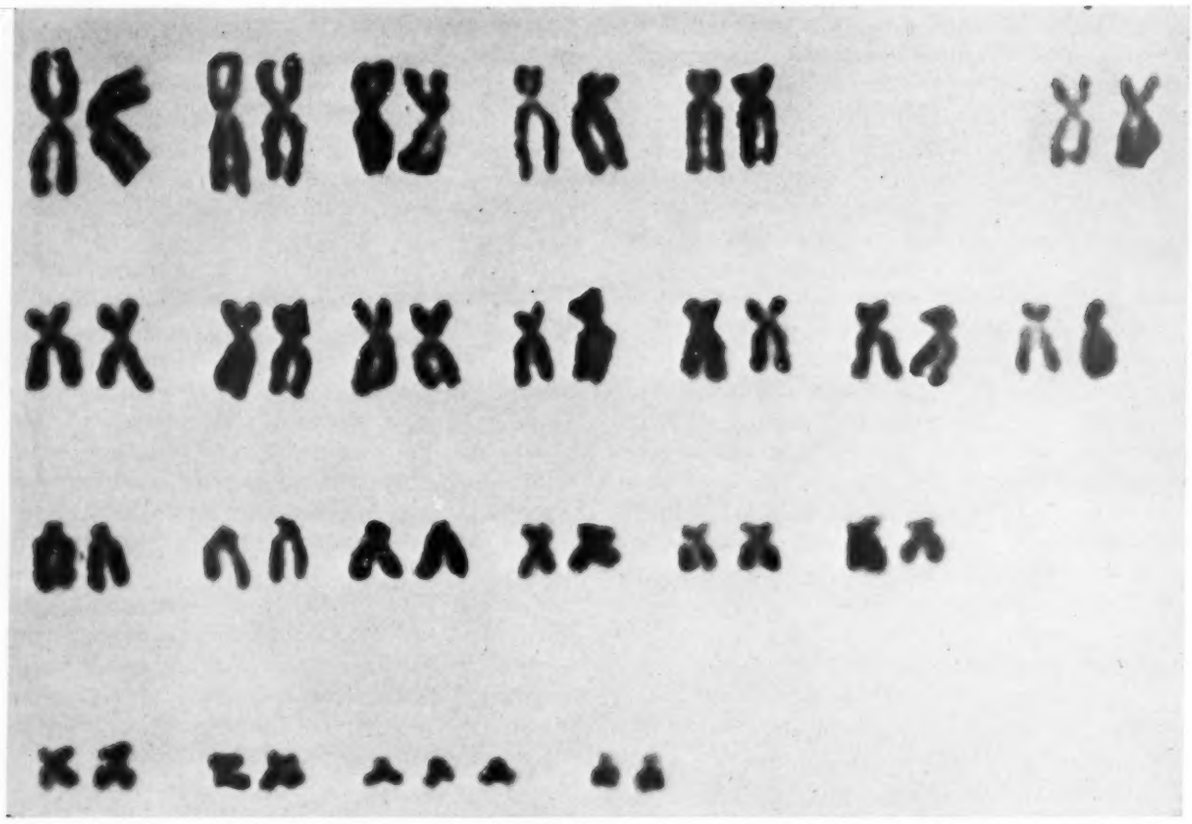


FIG. 8. Karyotype of mitotic chromosomes of a mongoloid idiot. Note trisomy of chromosome 21. Forty-seven chromosomes. Arranged according to the Denver system.

borne children. To our knowledge, seven such cases have been reported to date [*41–46*]. These mothers have produced mongoloid children more frequently than could be expected on the basis of a simple recessive type of inheritance. Two of the seven offspring of mongol mothers were also mongoloid, and another had some other type of mental retardation, probably of different origin. This strongly suggests a dominant type of inheritance. These contradictory facts indicate that some alteration must be present in the genetic material of mongoloid individuals which is expressed and transmitted as a dominant factor. This alteration must occur much more often than any single gene mutation is known to occur in man. Finally, the widespread abnormalities found in these individuals also suggest more than one gene effect, although this is not definite.

There are two types of alterations in genetic material that can produce changes in an organism and which can be transmitted to future progeny. One of these is mutation of single genes. The foregoing discussion suggests that mongolism is not explainable by the assumption of single gene mutations. The other type of genetic alteration is chromosomal aberration. In this case genetic material is altered by deficiency or excess of all or part of a chromosome, or by a positional effect due to rearrangement of its parts. In the case of mongolism, all the preceding facts are consistent with chromosomal aberration as the cause of the abnormality.

The chromosomal aberration in mongolism is now seen to be the presence of an extra chromosome. The result of this excess is to produce changes in almost every tissue and physical characteristic of the affected individual. Even the basic biochemistry of the mongoloid subject seems to be distorted, as evidenced by the abnormal glucose tolerance and basal metabolic rate these individuals exhibit [*47*].

The problem of how this extra chromosome arises is a key one, and may be elucidated by examining some basic facts well known to geneticists in the study of lower organisms. Since we are dealing with an abnormality of the chromosome number, it is necessary to under-

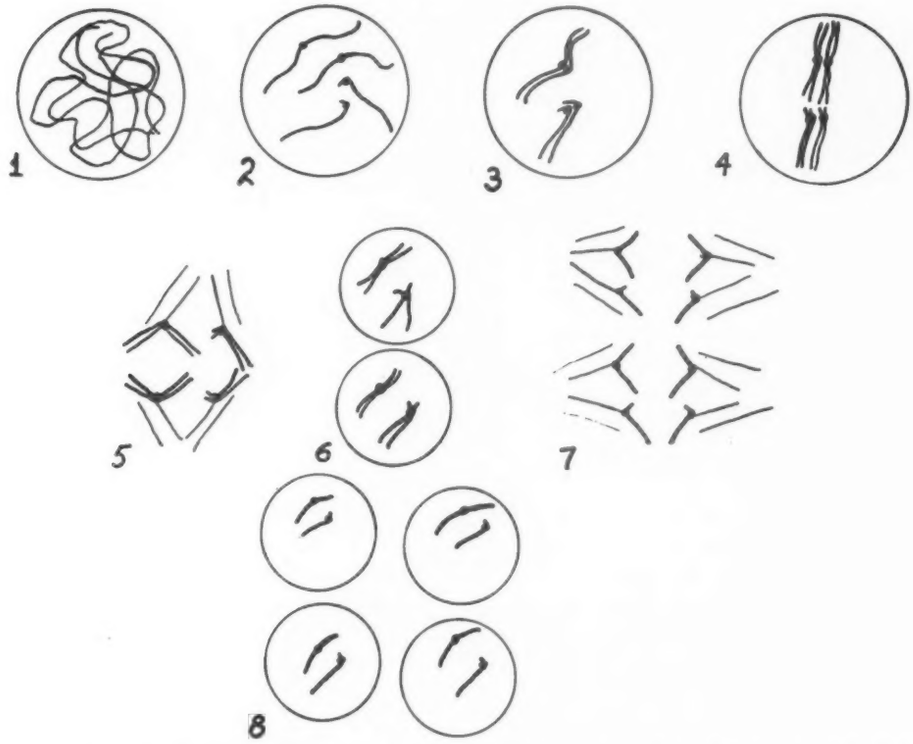


FIG. 9. Meiosis. 1, nucleus of primary gametocyte in prophase, leptotene stage. 2, pachytene stage. Individual chromosomes discernible. 3, synapsis. Members of each chromosome pair are associated. 4, diplotene stage. Members of chromosome pairs are seen to be reduplicated. Tetravalents arranged around equator of cell, nuclear membrane disappears, meiotic spindle appears. 5, diakinesis. Members of chromosome pairs are separated toward opposite poles (disjunction). 6, completion of first meiotic division. Two cells now present, each with half the diploid number of chromosomes present as divalents. 7, second meiotic division. Pairs of chromatids comprising each chromosome are separated (disjunction). 8, completion of second meiotic division. Four cells, each carrying the haploid number of chromosomes.

stand the process by which the human chromosome number is kept constant from individual to individual and from cell to cell.

At fertilization, the zygote is formed by the union of two cells, the ovum and the sperm, each of which contains, normally, one chromosome of each of the twenty-three pairs of chromosomes—the haploid number. These haploid cells arise from originally diploid (forty-six chromosomes) cells by the process of meiosis. The basic features of this process are (Fig. 9):

(1) Each chromosome in the nucleus comes to lie next to its homologue (other member of the pair). This is called synapsis, and is essential for normal meiosis. The X and Y in the male are partly homologous and therefore lie adjacent to one another, but are not completely synapsed as are the other chromosomes.

(2) Each chromosome is seen to be split longitudinally in two, forming two chromatids. The synapsis of such a pair of doubled chromosomes results in a four-stranded condition, a tetrad. It is at this stage, apparently, that crossing over occurs between the chromatids of the homologous chromosomes, resulting in new arrangements of genetic material and preserving genetic variability in the species.

(3) The first meiotic division occurs, in which the two doubled chromosomes in each tetrad are separated into different cells. This is the process of disjunction and is of vital importance to preservation of the chromosome number. The result of disjunction at the first meiotic division is to produce two cells, each containing one member of each of the twenty-three pairs of chromosomes in the form of a pair of chromatids.

(4) The second meiotic division then occurs, in which the two chromatids comprising each chromosome are separated and drawn into separate cells. This is disjunction at the second meiotic division and is also essential for proper maintenance of the chromosome number. The result is the formation from each primary germ cell of four cells, each containing the haploid

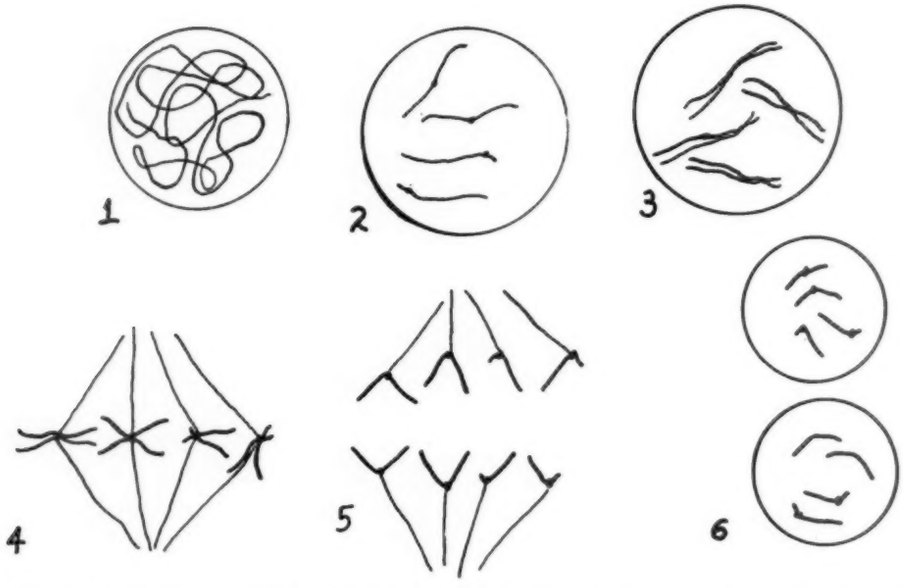


FIG. 10. Mitosis. 1, early prophase of somatic cell nucleus. 2, later prophase. Individual chromosomes apparent. 3, late prophase—early metaphase. Each chromosome is seen to be reduplicated. 4, metaphase. Nuclear membrane disappears. Chromosomes arranged on mitotic spindle. No association of homologous chromosomes. 5, anaphase. Two chromatids comprising each chromosome are drawn to opposite poles. 6, completion of mitosis. Two cells each containing the diploid number of chromosomes.

number of chromosomes. These are the gametes that will take part in fertilization. In the male, all four persist as spermatozoa; in the female, three of the products of meiosis degenerate into "polar bodies" while the fourth becomes a fertilizable ovum.

The new individual begins, then, as a diploid cell formed from two haploid cells, and possesses thereby the normal diploid number of chromosomes, forty-six, in the form of twenty-three matched pairs (or twenty-two pairs plus the X and Y in the male). From this one cell the new individual develops by repeated multiplications. The chromosome complement of each cell remains constant during this series of multiplications by means of the process of mitosis. In this process the chromosomes come to lie more or less centrally in the nucleus, but no synapsis occurs. Instead, the two chromatids of each chromosome are directly separated into different cells, thus producing two cells with the normal diploid number. (Fig. 10.) From the mechanical point of view, the basic difference between meiosis and mitosis is that in meiosis two cell divisions occur with only one chromosome replication, while in mitosis each cell division is accompanied by chromosomal replication.

With these facts in mind, one can explain the occurrence of an extra chromosome, i.e., a triad instead of a pair. It is fairly certain that every cell in the mongoloid's body contains this defect.* It appears, then, that the original zygote from which the mongoloid developed had the defect, and it was dutifully preserved by the process of mitosis. The original zygote must either have become defective after fertilization, or must have received a defective gamete from one of its parents. While there is no direct evidence from mongoloid material to support either of these hypotheses, information derived from the genetics of lower organisms supports the theory that a defect in one of the parental gametes is responsible. This defective gamete apparently possessed an extra twenty-first chromosome (i.e., two rather than one) and, on union with a normal gamete from the other parent, resulted in a zygote with three rather than two of the twenty-first chromosome. A probable explanation for the cause of such defective gametes comes from the work of Bridges [*49*]. He was observing the inheritance of sex-linked characters in fruit flies. It will be recalled that

* The process of cell division ceases in many tissues, such as skin, when the cells reach their mature state. Many cytologists believe that at the final mitosis before ceasing to divide, the cells may acquire abnormal chromosome numbers. This qualification to the preceding statement does not affect its general application [*48*].

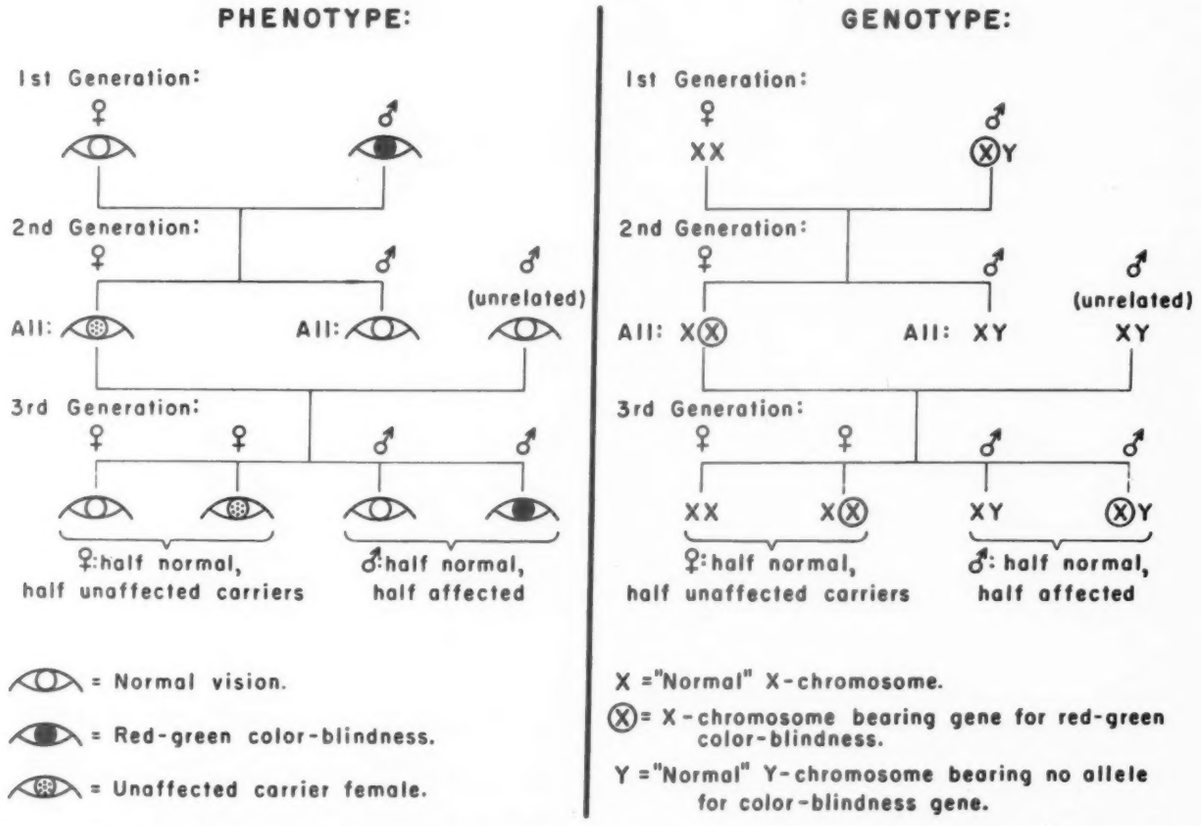


FIG. 11. Diagram illustrating recessive sex-linked inheritance. Red-green color blindness is taken as an example. Note that affected individuals are all males, sons of unaffected carrier females. These females generally are daughters of affected males, but may be the daughters of unaffected carriers. For a female to be affected, her father would have to be affected and her mother a carrier. Note that the trait follows the transmission of the X chromosome from the affected male.

the theory of sex-linked inheritance, as proposed by Morgan [*50*], concerns characters that follow a peculiar pattern of inheritance. A father who shows the character will produce all normal-appearing progeny. His sons produce only normal offspring. His daughters, although they themselves appear normal, will transmit the character to half their sons. Females rarely if ever show the character in question. Morgan postulated that the gene for a character showing this sort of inheritance pattern was located on the X chromosome and was a recessive. (Fig. 11.)

The following facts about sex determination should be understood. All normal ova produced by the mother contain an X chromosome. Two types of sperm are produced by the father: half carry an X chromosome, half carry a Y chromosome. If an ovum is fertilized by an X-bearing sperm, the zygote formed will be XX and will become a female. If fertilization is accomplished by a Y-bearing sperm, the zygote will be XY and will become a male.

It will be observed that a father transmits his Y chromosome to his sons and his X chromosome to his daughters, never the reverse. Males always receive their X chromosomes from their mothers. Thus, according to Morgan's theory, the sex-linked (or rather X-linked) gene will go from an affected father to all his daughters, who will carry the gene as a recessive, having a normal gene at the same location on the other X chromosome to offset its effect. His sons, receiving only the innocent Y from the affected father, will be entirely normal, having received their X from their normal mother. When the carrier daughters produce ova, half of them will carry an X chromosome bearing the normal gene, while half will carry an X chromosome bearing the abnormal gene. Thus, these daughters will transmit an affected X to half their sons, who will show the recessive trait because their Y will not

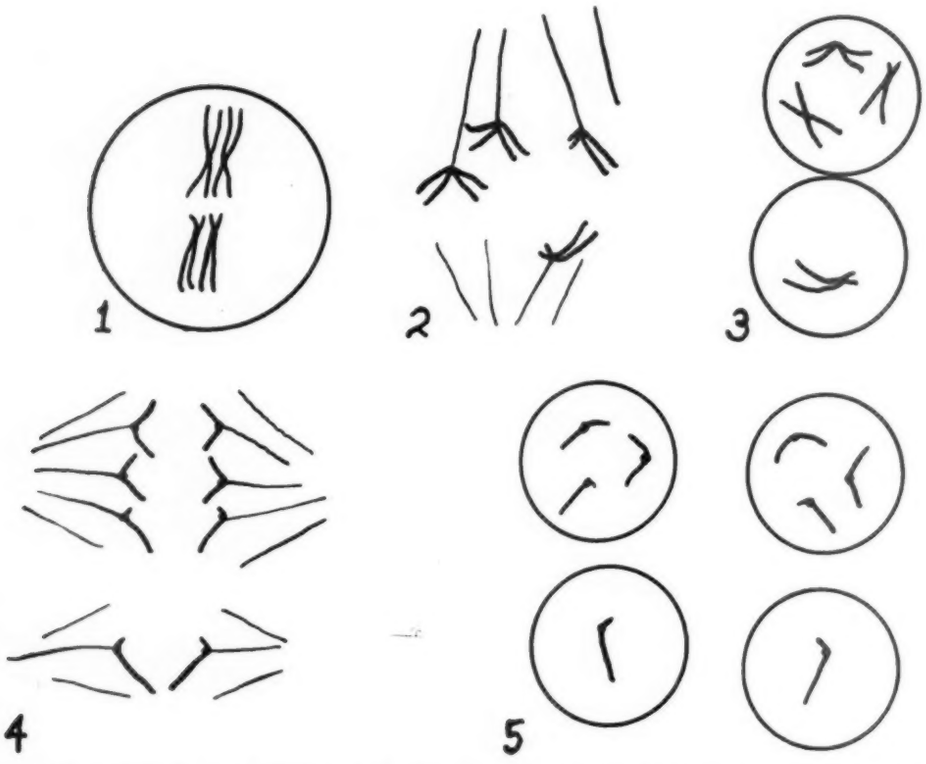


FIG. 12. Meiotic non-disjunction. 1, diplotene stage, meiotic metaphase. Synapsed pairs of homologous chromosomes. 2, non-disjunction at first meiotic division. Both members of one synapsed pair of chromosomes are drawn to the same pole instead of being separated. 3, completion of first meiotic division. One cell contains both members of one chromosome pair, the other lacks any member of that pair. 4, second meiotic division. 5, completion of second meiotic division. Half the gametes contain one more than the haploid number of chromosomes while half contain one less.

offset it. Similarly, half their daughters will again be carriers.

For a female to show a recessive sex-linked trait, she must carry two genes for the trait, one on each of her X chromosomes. This is easily arranged with drosophila in the laboratory. Bridges was working with female drosophila of this type. They carried two X-linked genes for the recessive trait, "white eye," and therefore showed the condition. When he crossed these white-eyed females to normal (red-eyed) males the vast majority of offspring were as predicted by Morgan's theory. The males, all receiving an abnormal X from their mother and a Y from their father, were white-eyed. The female offspring, all receiving an abnormal X from their mother and a normal one from their father, were red-eyed but could be shown by further breeding to carry the recessive trait.

Among many crosses of this type, however, on rare occasions white-eyed *females* and red-eyed *males* were produced, instead of the reverse. These occurred in about one of every 2,000 to 3,000 offspring of such a cross. Such an occurrence did not seem explainable by Morgan's theory. It meant, if Morgan were correct, that the white-eyed daughters did not receive a normal X from their father, but must have received two abnormal X's which could have come only from their mother. Likewise, the red-eyed sons must have received their father's normal X and not their mother's abnormal one. Bridges suggested a means whereby these irregularities could have occurred. He postulated that, in the formation of the ovum during meiosis, an accident had occurred. At the time of disjunction in the first meiotic division, the two synapsed X chromosomes had not been drawn into separate cells. Instead, *non-disjunction* had occurred and both X chromosomes migrated to the same pole. This resulted in an ovum with either two X chromosomes, or none at all, instead of the usual single X. (Fig. 12.) If the ovum contains two X chromosomes and mates with a normal Y-bearing sperm, it should produce an offspring with the unusual sex chromo-

some constitution of XXY. If mated with an X-bearing sperm, an XXX offspring was expected. The XXY offspring carried two abnormal genes for white eye, both obtained from the mother. Bridges predicted that the abnormal white-eyed females would have such an XXY constitution. When such females were subjected to cytologic examination it was indeed found that they carried an extra chromosome and that they did have an XXY constitution, thus confirming Bridges' brilliant series of predictions. Similarly, if the ovum that had received no X chromosome at all were fertilized with an X-bearing sperm the resultant offspring would be X-O in its sex determining chromosomal make-up, (i.e., it would have only one sex chromosome instead of two). Fertilization with a Y-bearing sperm would produce a Y-O individual. Bridges predicted that the unexpected red-eyed male flies he had found were these postulated X-O individuals. Again cytologic examination proved him correct. As for the XXX and YO individuals also predicted, some XXX's were found, but they were underdeveloped females and usually did not survive. Because of their three X chromosomes, Bridges dubbed them "superfemales" although there was nothing "super" about them in any way. As for YO individuals, none were found, and it is presumed that such zygotes were non-viable. This might be expected, considering the complete absence of any of the genetic material normally carried by the relatively large X chromosome.

The occurrence of non-disjunction in meiosis is thus seen to account for abnormal chromosome numbers, both greater and less than the normal number. In particular, here is a process that might produce three instead of two chromosomes of one type. That the process is not restricted to the sex chromosomes is shown by its widespread occurrence among plants. In general, being trisomic (having three rather than two of a given chromosome) results in decreased viability, as does being monosomic (only one chromosome instead of two). However, in the Jimson weed, which has twelve pairs of chromosomes, twelve variants have been found, each of which is trisomic for a different chromosome [*1*].

The process of non-disjunction, then, represents a mechanism by which the individual with mongolism may have come to possess an extra chromosome in every cell. While it does not tell us anything about the exact pathogenesis of the symptom complex, an understanding of this process at least provides us with insight into the basic defect in the condition. Mongolism thus appears to be the end result of a non-disjunction in the formation of one of the parental gametes. A gamete is produced that contains the normal haploid set of chromosomes plus an extra twenty-first chromosome. When this gamete mates with a normal one from the other parent, the resulting zygote is trisomic for the twenty-first chromosome, and the effect of this trisomy is to distort the development of the embryo and to produce the condition of mongolism.

It is generally believed that in mongolism the defective gamete most often is the maternal ovum. This is suggested by the correlation between the incidence of mongolism and advancing maternal age, while there is no such correlation with paternal age [*62*].

This effect of increasing age of the mother, but not of the father, led to the previous hypothesis of the etiology of mongolism—that of poor intrauterine environment in an older woman. Now that the primary defect in mongolism has been placed much earlier in time, during the formation of the ovum, the present hypothesis is suggested. Although there is some difference of opinion, it is believed that all of a woman's germ cells are already present in the oocyte stage while the ovary is still in its fetal state. These oocytes then remain dormant until adult life, when they divide to form the ova. Mintz [*51*] has recently suggested that at the time of birth the oocytes may have already begun the process of first meiotic division, and may finish dividing only shortly before ovulation. This is quite different from the situation in the male in whom new spermatocytes are constantly formed from puberty on. If aging of a cell is capable of causing damage to the normal spindle mechanism, thereby predisposing to the occurrence of non-disjunction, the aging maternal germ cells have a far greater chance of becoming abnormal than do the paternal germ cells, which are constantly being newly produced.

CHROMOSOME STUDIES IN INTERSEXES

As mentioned earlier, application of the technic of sex chromatin examination produced unusual findings in the majority of patients with Klinefelter's syndrome. These individuals while apparently male, were found to have nuclear chromatin bodies characteristic of female tissue. The incidence of Klinefelter's syndrome has been estimated to be as high as one in 1,000 to 3,000

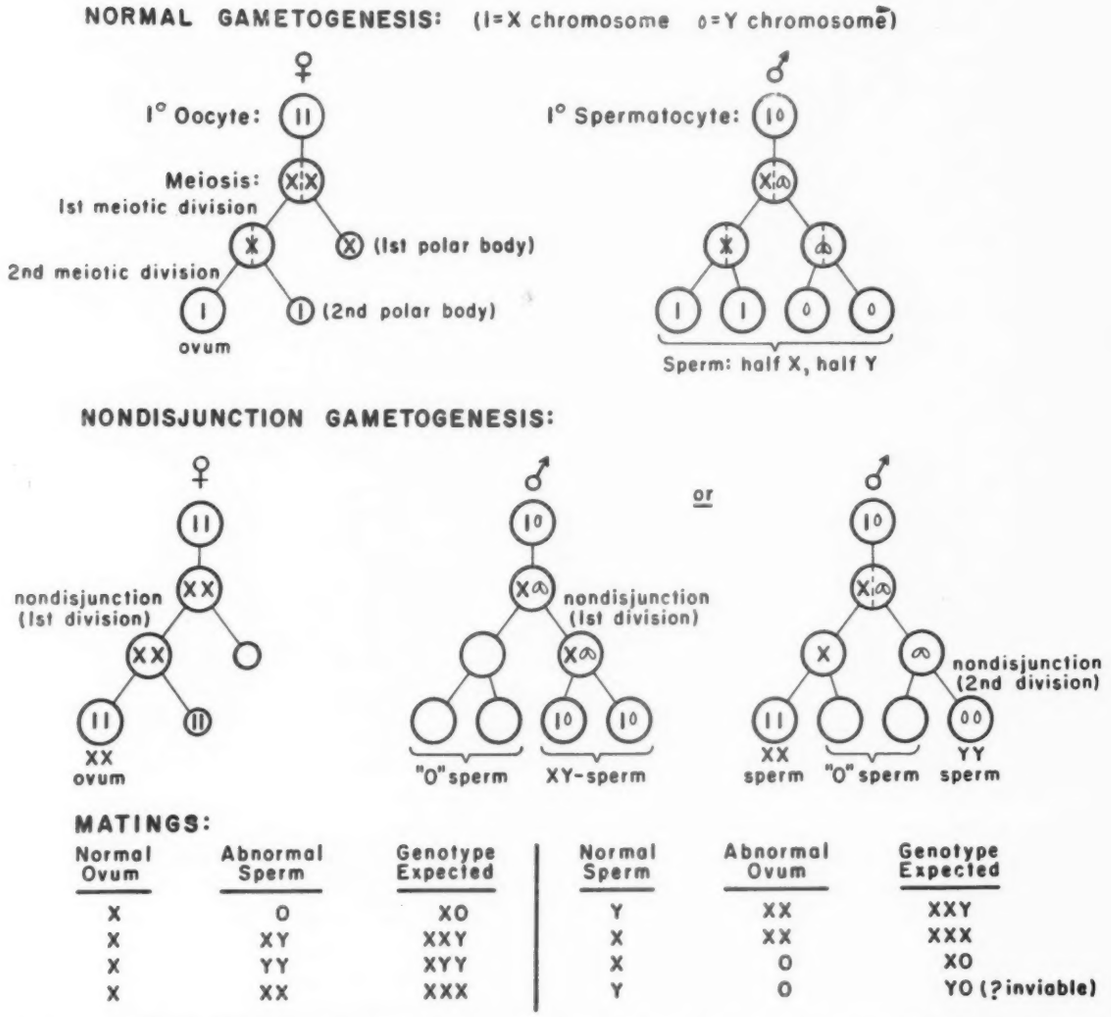
FIG. 13. Karyotype of mitotic chromosomes from an individual with Klinefelter's syndrome. Note the presence of two X chromosomes together with a Y chromosome. Forty-seven chromosomes. Arranged according to Denver system.

live male births in the general population [*52–54*]. Among mentally defective subjects, however, the incidence may be as high as 1 to 3 per cent [*52,54–56*].

Speculation as to the cause of Klinefelter's syndrome led to analysis of the incidence of the X-linked condition of red-green color blindness in patients with this condition. Polani et al. [*57*] found an incidence of color blindness among such individuals that suggested the presence of two X chromosomes. The following year Jacobs and Strong [*58*] reported such a case in which they had counted the chromosomes in bone marrow cells. They found a chromosome number of forty-seven. There were eight pairs of metacentric chromosomes in the medium size range, indicating the presence of two X chromosomes. However, there were also five small acrocentrics, indicating the presence of a Y chromosome. (Fig. 13.) The patient had, apparently, a sex chromosomal constitution of the XXY type, and was phenotypically a male. Subsequent workers have confirmed this finding [*59,60*] and it is now accepted that most patients showing the Klinefelter syndrome have this chromosomal aberration.

It is of interest to observe that the XXY individual found among drosophila by Bridges was a fully viable, fertile female, while in the human an XXY individual is a sterile male. This fact has important implications in the general genetic theory of sex determination, and will be discussed later.

In the Klinefelter syndrome we have another situation in which an extra chromosome occurs in every cell examined (allowing for occasional different counts due to technical errors). In mongolism there is also an extra chromosome, one of the small acrocentric autosomes. (An autosome is any chromosome other than the X or Y.) The patient with Klinefelter's syndrome, however, has an extra chromosome of the sex-determining pair. The origin of the extra chromosome here, as in mongolism, is thought



MATINGS:

Normal Ovum	Abnormal Sperm	Genotype Expected	Normal Sperm	Abnormal Ovum	Genotype Expected
X	O	XO	Y	XX	XXY
X	XY	XXY	X	XX	XXX
X	YY	XYY	X	O	XO
X	XX	XXX	Y	O	YO (?inviable)

FIG. 14. Diagram illustrating the formation of normal gametes and the types of gametes resulting from non-disjunction involving the sex chromosomes. Predicted results from matings involving such abnormal gametes and normal gametes from the opposite sex are shown.

to be non-disjunction. Thus, at meiosis, abnormal gametes are produced as a result of non-disjunction of the sex chromosomes. In the female, this would produce either an XX ovum or an "O" ovum devoid of sex chromosomes. In the male, either an XY sperm or an "O" sperm would be produced. There are two ways in which an XXY constitution might arise, depending on which parent produced the abnormal gamete. (Fig. 14.) These are either fertilization of an XX ovum by a normal Y sperm, or of a normal X ovum by an XY sperm.

In mongolism, there is good evidence to support the idea that most cases arise through maternal rather than paternal non-disjunction. However, in Klinefelter's syndrome the evidence pointing toward one or the other parent as the more frequent cause is less definite. Stern [*61*] has analyzed data on the incidence of red-green color blindness in these patients and on statistical grounds believes that the most common causative event is maternal non-disjunction. As yet no correlation with maternal age has been reported.

TURNER'S SYNDROME

From the foregoing discussion it seems probable that XO individuals as well as XXY ones will be produced as a result of non-disjunction of the sex chromosomes. The possibility of an XO constitution in human subjects had been postulated in 1956 by Polani et al. [*63*] after an analysis of the incidence of color blindness in females with the Turner syndrome. These individuals were usually found to have negative

FIG. 15. Karyotype of mitotic chromosomes from an individual with Turner's syndrome. Note the presence of only one X chromosome. No other sex chromosome is present. Forty-five chromosomes. Denver system.

sex chromatin patterns and a male incidence of red-green color blindness. On the basis of these findings Polani postulated the presence of only one X chromosome in these patients and possibly an XY or XO constitution. In 1959 Ford et al. [*64*] reported the first chromosome analysis of a case of Turner's syndrome and verified Polani's suggestion that these patients have only one X chromosome. The chromosome number in their patient was forty-five and the sex chromosome constitution was XO. (Fig. 15.) The patient's karyotype revealed an unmatched medium-sized metacentric chromosome which was interpreted as a single X chromosome. There was no corresponding small acrocentric or Y chromosome. Thus only one member of the sex determining pair, a single X, was present. This finding was confirmed by other investigators [*65,66*] shortly thereafter, and it is now considered established that Turner's syndrome is the result of a sex chromosome constitution of the XO type.

The Turner syndrome, then, may be classed with mongolism and the Klinefelter syndrome as a disease due to an abnormality of the chromosome number. The cause of these abnormal chromosome numbers most probably is non-disjunction during the production of parental gametes. Again, as in Klinefelter's syndrome, there is no definite evidence as to whether maternal or paternal non-disjunction is the more frequent cause of Turner syndrome.

It should be observed that, among drosophila, XO individuals are sterile males, while among human beings they are sterile females. In addition, the human XO condition is associated with a variety of other congenital malformations, while the drosophila XO suffers only from sterility.

THE SUPERFEMALE

It will be recalled that, as a result of the fertilization of a non-disjoined XX ovum by an X sperm, Bridges found a class of female flies that had reduced viability and were physically below normal. Because of the XXX constitution he called them *superfemale*. If Klinefelter's and Turner's syndromes really are due to parental non-disjunction and the resultant abnormal chromosome number, extension of the theory

suggests that XXX human subjects should also exist.

In 1959 Jacobs et al. [*67*] described a sexually infantile female who had scanty menses which eventually ceased altogether. This patient had a positive sex chromatin pattern and even showed two nuclear chromatin bodies in most of the cells of a buccal mucosa scraping. Examination of her chromosomes revealed that she had a chromosome number of forty-seven with a karyotype indicating three X chromosomes. Jacobs et al. have recently discovered another case of the same type [*68*]. The discovery of these cases, whose existence was predicted by theory, is the strongest confirmation of the correctness of the theory. It may therefore be stated with some confidence that the Klinefelter syndrome, the Turner syndrome and the superfemale represent the human counterparts of the XXY, XO and XXX in drosophila, and that the etiology of the chromosomal abnormality is the same in both cases.

In a recent survey conducted among institutionalized mentally defective subjects, Ferguson-Smith studied two individuals whose buccal smears showed two sex chromatin bodies in each nucleus [*69*]. These individuals were phenotypically males, but had forty-eight chromosomes, with forty-four autosomes (normal) and a sex chromosome constitution of XXXY type. The mode of origin of such a condition is uncertain. Non-disjunction may have occurred by chance in both parents, or an XXX ovum may have been produced by the mother because of non-disjunction at both first and second meiotic divisions.

CHROMOSOMAL ABERRATIONS IN OTHER CONDITIONS

Just as the identification of an XXX condition in man provided confirmation of previous postulations, the discovery of another type of chromosomal aberration has given further evidence of the specificity of the defects produced by such aberrations.

In 1959 Ford et al. [*70*] described a patient who exhibited clinically the features of both mongolism and the Klinefelter syndrome. As might be expected this patient's karyotype showed trisomy for the twenty-first chromosome and an XXY sex chromosome pattern. The chromosome number was forty-eight. In our laboratory we have examined the chromosomes of a similar patient [*71*] with the same results. (Fig. 16.) It would seem that trisomy for the twenty-first chromosome produces mongolism regardless of the condition of the other chromosomes; the same may be said for the specificity of the XXY condition. However, the complete specificity of such abnormalities of chromosome number is doubtful, in view of a recent report by Fraccaro et al. [*72*]. These workers describe a patient with mongolism with forty-six rather than forty-seven chromosomes, but only one twenty-first chromosome. One of the forty-six chromosomes was an unmatched small submedian, resembling the normal chromosome nineteen. At first it was postulated that the patient did have trisomy for the twenty-first chromosome, but that two of the three chromosomes had become translocated and fused into one to form the extra submedian that was found. However, examination of the chromosomes of the normal father of the patient revealed that this man was trisomic for the nineteenth chromosome. At present it cannot be decided whether the original explanation is the true one, or whether the mongol child is in fact trisomic for the nineteenth chromosome like his father, and is monosomic for the twenty-first chromosome. If this is the case, it would seem that the imbalance caused by absence of a twenty-first chromosome is phenotypically indistinguishable from that caused by the presence of an extra one. It is also evident that trisomy for the nineteenth chromosome is a benign condition producing no easily demonstrable abnormalities. A preliminary report by Böök describes a triple nineteenth chromosome [*73*]. This patient's only apparent abnormality was an interatrial septal defect. The patient's son was also examined, and was found to have the same abnormalities as his mother—trisomy for the nineteenth chromosome and interatrial septal defect.

Several other trisomic states have been reported. Edwards et al. [*74*] recorded a case of multiple congenital anomalies with trisomy of the seventeenth chromosome. Patau et al. [*75*] have described two cases of congenital abnormalities in which there was trisomy for one of the medium-sized acrocentrics (i.e., number 13, 14 or 15). They do not believe they can determine which of these three very similar chromosomes is actually trisomic. Patau et al. [*76*] have also found six cases of another group of congenital anomalies in which there was trisomy for the eighteenth chromosome. The triple 17 of Edwards and the triple 18 of Patau seem to be the same entity. It is difficult to differentiate 17

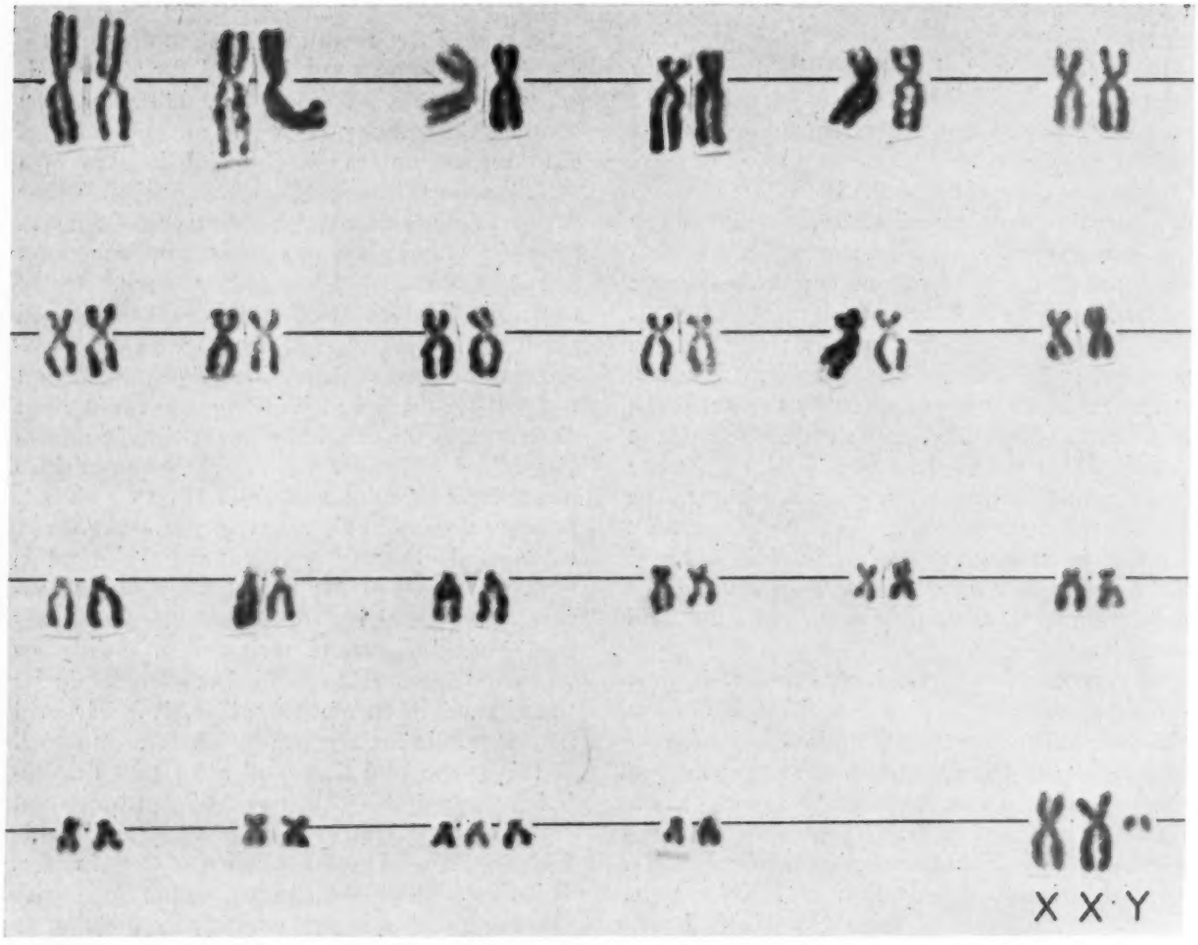


FIG. 16. Karyotype of mitotic chromosomes from an individual with both mongolism and Klinefelter's syndrome. Forty-seven chromosomes. Note trisomy for chromosome 21 and XXY sex chromosome constitution. Arrangement used is one applied prior to the Denver meeting.

and 18 with certainty. Fraccaro et al. [*77*] have described a patient who had forty-nine chromosomes and exhibited facial and cephalic peculiarities and an intersexual state. This individual was thought to be trisomic for the eighth and eleventh chromosomes with either an XXY sex chromosome constitution or a triple twenty-second chromosome with XX constitution. It is now thought to be a case of XXXXY with normal autosomes [*103*]. Finally, Böök and Santesson [*78*] have described an individual with mental retardation and disease of the central nervous system who has sixty-nine chromosomes. This patient is a complete *triploid*, i.e., he has three of each chromosome instead of the expected pair. Further work has shown that this subject is an example of mosaicism (see Mosaicism, p. 461) with both normal and triploid cells present [*104*].

Undoubtedly other trisomic syndromes will be described in the near future. It is probable that trisomy for any of the twenty-three chromosome pairs, and combined states with any two or more chromosomes present in triplicate will also be found. Some of these situations doubtless will be lethal and therefore will have to be discovered in aborted fetuses, but it is apparent from the preceding group of cases that it is impossible to predict the extent of the abnormality that any given trisomic state will produce.

The fact that trisomy for the nineteenth chromosome is compatible with normal development is especially important. It suggests that the abnormalities found in mongolism are not the result of the imbalance produced by an excess of a whole chromosome's content of genetic material, but only of such imbalance at one or a few key gene loci. Such a possibility

brings mongolism closer to the class of diseases caused by single gene mutations which produce specific biochemical abnormalities. Examples of such conditions are phenylketonuria and galactosemia, both of which produce severe mental deficiency. In both of these conditions the basic biochemical defect is fairly well understood and attempts at treatment are being met with some success [*79*]. The importance of these speculations lies in the possibility that an underlying biochemical defect in mongolism, due to the action of one or a few genes on the twenty-first chromosome, may yet be uncovered. If such proves to be the case, mongolism too may eventually become a treatable disease. Recent work by Jérôme et al. [*105*] suggests an accelerated pathway in tryptophane metabolism.

TRANSLOCATION

All of the conditions thus far discussed involve an excess or deficiency of at least one entire chromosome. Another type of chromosomal aberration may occur and may be associated with disease. In this case, the aberration involves parts of chromosomes rather than whole chromosomes. During the course of mitosis (or meiosis) chromosomes may become fragmented. Such chromosome fragments frequently become joined to other fragmented chromosomes, producing a "new" chromosome. Such alterations are called *translocations*. Turpin et al. [*80*] were the first to report such an occurrence in man. Their case presented a child with multiple abnormalities of the spine, and chromosome analysis revealed the apparent absence of one of the small and one of the large acrocentrics. The chromosome number was forty-five. Careful study suggested that the missing short chromosome had been translocated and was attached to the short arm of one of the longer acrocentrics. It is probable that some chromosomal material was lost, since translocations usually take place between the broken ends of two chromosome fragments. Also, it is questionable whether any disease would have been produced if the only abnormality were the attachment of two intact chromosomes.

Polani et al. [*81*] have found a case of mongolism in which the extra chromosome characteristic of that disease was translocated and attached to one of the longer acrocentrics. The chromosome number was apparently normal, forty-six. However, the discovery of the translocated extra chromosome indicated again that mongolism occurs when the genetic material of the twenty-first chromosome is present in "triple dose."

Another case, found by Lejeune et al. [*82*], was a patient with Klinefelter's syndrome who had only forty-six chromosomes instead of the usual forty-seven. On careful analysis two of the six medium-sized acrocentrics (possibly one number 13 and one number 15) were found to be missing. Their place had apparently been taken by a single long median chromosome. It is believed that this long chromosome is actually made up of both of the missing acrocentrics, translocated end to end.

MOSAICISM

During the development of a new individual from a zygote a series of mitotic divisions, each involving replication of the whole chromosome set, occurs. It occasionally happens that a chromosome becomes translocated or lost altogether during mitosis in the early stages of growth. This might result in a portion of the body containing normal cells and another portion containing replications of the abnormal cell. Such a condition is referred to as *mosaicism*.

The occurrence of mosaicism due to a chromosomal aberration in man was suggested by Ford et al. [*83*] when they reported the findings of a chromosomal analysis of a case of Klinefelter's syndrome. The majority of bone marrow cells examined showed forty-seven chromosomes with XXY sex chromosome constitution. However, a significant number of cells, more than could be attributed to technical error, were of XX constitution. It was suggested that this was due to mosaicism, and that the Y chromosome had been lost from one of the earliest cells of the embryo, resulting in a significant proportion of XX cells. Such loss of a sex chromosome has been known among drosophila and is one cause of so-called gynandromorphs, where part of the body is female (XX) and part "male" (XO) [*1*]. A similar case has been reported by Crooke and Hayward [*84*].

Studies of a patient at our laboratory revealed a related type of abnormality [*85*]. In this instance an infant was born with female genitalia and a much enlarged clitoris, or small phallus. Sex chromatin studies yielded consistently negative (male) patterns. Laparotomy revealed the presence of a uterus and fallopian tubes with two gonads. Histologic sections of these gonads disclosed a grossly disordered structure, but most pathologists agree that both ovarian and

testicular elements are present. It is probable that this patient is a true hermaphrodite.

Analysis of the chromosomes showed two types of cells in the bone marrow. About 40 per cent had an XY constitution with a Y chromosome that seemed somewhat larger than usually found. (In our experience, as well as in the experience of other workers [*86*], the Y chromosome is more variable in length than the other chromosomes.) The remaining cells showed an XO constitution. It is probable that in this case the Y chromosome was lost in one of the early cells of the embryo, producing the picture seen. The extent and distribution of the mosaicism is not yet known, since the proportion of atypical cells in the bone marrow may not reflect the distribution throughout the body.

To date, chromosomal analyses of two other cases of true hermaphroditism have appeared in the literature [*87,88*]. One of these was phenotypically female, while the other was an apparent male. Both showed the presence of an ovo testis, confirming the diagnosis of true hermaphroditism. On chromosome analysis both showed the XX sex chromosome constitution. The origin of partly functional testicular tissue in these individuals is problematical, and it has been suggested that such patients are actually mosaics. The finding of only one cell type in the bone marrow, skin and peripheral blood in these cases does not rule out the presence of a mosaic in the gonadal tissue. The case of XY/XO mosaicism just described supports the idea that true hermaphrodites may be mosaic individuals. In the cases in which only XX cells were found it is possible that a mosaicism of the XX/XXY or XX/XO type actually exists but has not been detected.

As already implied, other types of sex chromosome mosaics may occur, and some have been found. Ford [*59*] and Fraccaro et al. [*89*] have found cases of mosaicism of the XX/XO type in which some of the cells are normal (XX) and some have only a single sex chromosome (XO). These patients all presented clinically with Turner's syndrome, but were sex chromatin-positive. Such mosaicism may account for some of the cases of chromatin-positive Turner's syndrome that have been reported.

Jacobs et al. [*68*] have studied another interesting type of mosaic. This patient had a primarily XXX sex chromosome constitution in cells cultured from skin, while the peripheral blood and bone marrow were largely of XO constitution. The case came to their attention because of the presence of double sex chromatin bodies in buccal mucosa scrapings (related to the XXX constitution). The peripheral blood, however, showed no "drumsticks" (related to the XO constitution).

In the cases of mosaicism just described, the individual's body was found to be composed of two types of cells. At least one of these cell types possessed an abnormal sex chromosome constitution (XXY, XO or XXX). It is apparent that these atypical cells can be responsible for abnormal sexual development despite the presence of a large number of normal cells in the body. The exact location of the abnormal cells is probably critical. If they comprise a large proportion of the gonadal tissue, abnormalities of sexual development may be expected.

Court Brown et al. [*90*] have discussed the subject of mosaicism and suggest certain criteria which should be met in order to diagnose mosaicism by chromosome counts: (1) the frequency of the cells with non-modal chromosome number should be greater than that expected by chance alone; (2) the determination should be made on repeated specimens from different tissues, if possible; and (3) all the non-modal cells should have the same karyotype.

It is to be expected that mosaicism for autosomes will also be discovered, but to date none have been reported.*

ISOCHROMOSOMES

Another chromosomal anomaly has been mentioned by Fraccaro et al. [*91*]. They have found three patients who present some of the features of Turner's syndrome (primary amenorrhea, short stature, elevated urinary gonadotrophins) but lack others (no webbed neck, sex chromatin-positive). These patients all had forty-six chromosomes, but the chromosomes thought to be the XX pair did not match one another. One was considerably longer than the other and, unlike the usual X chromosome, had a perfectly median centromere. Fraccaro postulates that this chromosome arose by the splitting of the centromere the wrong way at the second meiotic division. Instead of splitting vertically down the middle, thereby separating the two chromatids, the centromere split horizontally. The long arms of both chromatids remained joined and went to the same daughter cell,

* See Addendum.

while the short arms went to the other. The long arms then behaved as a new chromosome and were replicated as such at each cell division thereafter. This new "isochromosome" carries none of the genetic material of the short arm of the X chromosome and a double dose of the material of the long arm. It is possible that the genetic material in the short arm of the X chromosome is required in double dose for normal female development. Its absence, either in the XO individual or because of a long arm isochromosome, results in primary amenorrhea, with or without the complete Turner's syndrome.

COMMENTS

It is apparent that significant contributions to medical knowledge have been made by the application of the technics of cytogenetics to human disorders. The basic defect and its probable mode of origin have been discovered for three well known diseases: mongolism, Klinefelter's syndrome and Turner's syndrome. In addition, one possible etiology for multiple congenital anomalies and intersexes is now recognized, and merits investigation in appropriate cases. While the frequency of chromosomal aberration as a cause of these conditions is not known, further investigation will undoubtedly produce many such cases. In particular, chromosomal analysis may clarify the pathologic condition in individual cases. Thus Harnden and Stewart [*92*] have reported a case of "pure gonadal dysgenesis" in an apparent female. This patient was tall, thin and sexually undeveloped, but otherwise had none of the stigmata of Turner's syndrome. The patient had a vagina, uterus and fallopian tubes, and artificial menses occurred following estrogen treatment. Cases of this sort have been generally considered to be variants of Turner's syndrome, lacking the short stature, webbed neck and other recognized characteristics of the disease, but exhibiting the major defect of secondary sexual and gonadal underdevelopment. Harnden and Stewart's case, however, on chromosome analysis was shown to be of XY constitution, i.e., a male karyotype, unlike the XO usually found in Turner's syndrome. This case emphasized the importance of considering more than the chromosomes alone in trying to understand the development of the human phenotype. The patient in question, while chromosomally a male, developed as a female either because of intrauterine hormonal influences or perhaps as a result of some rare dominant single gene mutation. A possible explanation of this case is suggested by the work of Jost [*93,94*]. He has shown in rabbits that males castrated in utero developed the female phenotype, presumably because of maternal hormonal feminizing effects. In the case under discussion, as a result of some genetic factor or of some intrauterine accident, it is possible that the fetus did not develop any functioning testicular tissue. It was effectively castrated, and the result was the same as in Jost's rabbits.

This case should not be confused with the syndrome of testicular feminization, in which XY sex chromosome constitutions have also been found in apparently female individuals [*95*]. These patients, unlike Harnden and Stewart's patient, have undescended testes and no uterus or tubes. The vagina ends as a blind pouch of varying length and the clitoris is of normal size. This condition is inherited as a single gene defect [*95*].

The foregoing speculations bring up the knotty problem of sex determination, which until recently was thought to be fairly well settled by the work of Bridges [*96*]. It will be recalled that among drosophila, flies of XXY constitution are normal females, while flies of XO constitution are healthy but sterile males. Thus the XXY and XX conditions are indistinguishable, and the XY and XO conditions differ only in respect to sterility. The presence of two X chromosomes makes the fly a female, and an added Y has no effect. If one of the two X's is "subtracted," the result is a male. Apparently the presence of two X chromosomes provides enough female-determining genetic material to produce a female fly, while one X chromosome does not, its effect being overcome by male-determining genes which have been shown to be carried on the autosomes [*96*]. The "female" genes on the X chromosome are sufficiently potent in double dose to overcome the effect of a diploid set of male-determining autosomes, and produce a female. A single X, however, does not provide sufficient feminizing influence to overcome a diploid set of male-determining autosomes, so that the XY or XO drosophila is a male. This explanation is known as the "balance theory" of sex determination.

Thus the Y chromosome appears to have little or nothing to do with the production of male characteristics in the drosophila. However, in man this obviously is not the case, since the XO individual is a female and the XXY a male.

Seemingly, the addition of a Y chromosome to a female (XX) constitution converts it to a male phenotype. Similarly, the lack of a Y chromosome (XO) is not insignificant and any autosomal male sex determiners are apparently unable to overcome the effect of a single X chromosome. These facts suggest that, in man, potent male determiners are linked with the Y chromosome, since maleness apparently cannot occur in its absence, and its presence produces maleness even when two X chromosomes are also present.

Similarly, Welshons et al. [*97*] have shown in mice that the XO condition produces a female which, unlike the human XO, is usually fertile. It is of interest that Bahner et al. [*98*] have described a woman with XO sex chromosome constitution who bore a child, showing that the XO human being may also be fertile in rare cases. Thus it seems that among the mammals that have been studied the Y chromosome is a bearer of male sex determiners.

It would appear then, at first glance, that the balance theory of sex determination has been shown to be non-applicable for mammals, but that the X and Y chromosomes are themselves the bearers of the necessary sex determinants. However, it is important to bear in mind the fact that placental mammals do not develop independently of maternal sexual effects, as drosophila do. A well known example of the effect of the intrauterine hormonal environment is the masculinization of female fetuses by administration of progesterone to the mother during pregnancy [*99*]. As shown by Jost, a castrated mammalian embryo develops the female phenotype, possibly as a result of endogenous maternal feminizing secretions [*94*]. In order to combat this feminizing effect, functioning embryonic testicular tissue seems necessary. The presence of a Y chromosome is apparently required for the development of such testicular tissue, since the human XO individual usually has little or no gonadal tissue. It would seem that such an individual, without functioning testicular tissue, develops as a female because of the combined effects of the single X chromosome and the maternal hormonal milieu. Any autosomal male determiners are apparently overwhelmed. In the same way, in the XXY human being the addition of the Y chromosome results in the production of apparently functional testicular tissue, and masculinization occurs despite a double dose of X chromosome female determiners and the maternal hormonal milieu. It cannot be confidently stated that this is because the Y chromosome is the sole factor involved in male determination. It may be that addition of the Y chromosome to the autosomal male determiners already present is sufficient to tip the balance in favor of maleness.

It is of interest in this connection that the previously mentioned patient of Böök's with complete triploidy (sixty-nine chromosomes) [*78*] seemed to have normal sexual differentiation. His sex chromosome constitution was of the XXY type, but he differed from a patient with Klinefelter's syndrome by having an extra set of autosomes. The presence of this extra set of autosomes may have increased the "maleness" of the patient. It can be tentatively postulated, on the basis of this information, that the autosomes do contain masculinizing factors.

Thus, with present evidence, the balance theory of sex determination cannot be considered definitely to be inapplicable to mammalian sex determination. With the modifications indicated, it may still be valid.

SUMMARY

A brief history of the development of modern technics in human cytogenetics is presented, and application of these technics to the study of human disease is illustrated.

The basic chromosomal defects in several disease states are described. The best studied conditions to date are mongolism, Klinefelter's syndrome and Turner's syndrome. In mongolism there is trisomy for the twenty-first autosome. In Klinefelter's syndrome there is an XXY sex chromosome constitution. In Turner's syndrome there is an XO sex chromosome constitution. The chromosome numbers in these conditions are, respectively, forty-seven, forty-seven and forty-five, whereas the normal human chromosome number is forty-six.

Non-disjunction of chromosomes at meiosis, in the production of parental gametes, is postulated to be the mechanism for the production of the chromosomal aberrations in the three conditions mentioned. The process of non-disjunction is discussed in detail. It results in the production of parental gametes with either more or less than the normal haploid number of chromosomes. Fertilization involving such a gamete and another normal one may result in a zygote that is either trisomic for the chromosome involved or is deficient in one chromosome.

Other types of chromosomal aberrations are considered. These include translocation, mosaicism, and isochromosome formation. The bearing of recent information on the theory of sex determination is also discussed.

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APPENDIX

The methods and materials used by the authors for chromosome preparations vary with the tissue to be examined. We shall describe the ones used for bone marrow, skin and peripheral blood.

Bone Marrow. Approximately 1 cc. of bone marrow is aspirated under sterile conditions from the sternum or iliac crest and suspended in 3 cc. of nutrient medium, consisting of 60 per cent medium 199 (Difco), 35 per cent single donor AB serum and 5 per cent freshly prepared chicken embryo extract. The medium also contains 200 units per cc. penicillin, 100 μg. per cc. streptomycin and 20 units per cc. Mycostatin® (Squibb, suspension). This is then distributed into sterile 60 mm. Petri dishes containing a cover slip, with 1 cc. in each Petri dish. To each dish is added 2 to 3 cc. of the nutrient medium and the covered dishes are placed in a 37°c. incubator in a 5 per cent CO_2 atmosphere. Depending on the cellularity of the original innoculum, the dishes are removed from twenty-four to seventy-two hours later, the higher cell concentrations requiring less time. No colchicine is used in this method. The cover slips are removed with sterile forceps, and the dishes returned to the incubator for eventual passage of the cells. The cover slips are placed in 0.7 per cent sodium citrate for ten minutes, and then in 2 per cent acetic orcein (2 gm. natural orcein [Matheson, Coleman and Bell], dissolved in 45 cc. glacial acetic acid by heating with a reflux condenser, then add 55 cc. distilled water while still warm; filter before use) for five minutes. The cover slip is inverted on a clean slide, squashed gently and examined. If spreading is insufficient, one may squash more strongly. Seal with Kronig's cement. This method is a modification of that described by Fraccaro, Kaijser and Lindsten [*31*].

Skin. After injection of 1 per cent procaine deep into the area to be biopsied (usually the posterolateral aspect of upper thigh or buttock), not intradermally, a superficial fold of skin is grasped with pointed tissue forceps and is cut with a sharp scalpel blade, under sterile conditions. The resulting fragment is usually approximately 5 by 4 mm. and is 2 mm. thick. This procedure leaves no scar. The skin is kept in sterile saline solution until preparation. The fragment is cut into small explants (approximately 1 by 1 mm.), which are distributed into two or three 60 mm. Petri dishes with cover slips, some pieces lying on and some off the cover slip. No plasma clot is required. Nutrient medium, consisting of 70 per cent medium 199, 20 per cent single donor AB serum and 10 per cent chicken embryo extract, with the same antibiotics as in the bone marrow medium, is added in amount not quite sufficient to cover the explants (usually 1.5 cc.). The covered dishes are incubated at 37°c. in a 5 per cent CO_2 atmosphere. The medium is changed three times a week without disturbing the explants. After one to three weeks, a monolayer of cells has grown from the explants onto the glass, and a deeper layer of medium (3 cc.) may be used. When growth appears to be active, the cover slips are treated as in the bone marrow technic. Since these preparations will usually not give many mitoses for study, the remaining cells are removed from the Petri dishes by incubating for twenty minutes with 2 cc. of a 50/50 mixture of trypsin and Versene,® made up in calcium- and magnesium-free Hanks' solution. The resulting cell suspension is centrifuged gently for five minutes (400 to 500 r.p.m.), resuspended in 2 to 10 cc. of the nutrient medium, the amount depending on the cell density. Two cubic centimeter aliquots of the suspension are placed in 60 mm. Petri dishes with cover slips

and replaced in the CO_2 incubator. After three to four days a heavy growth of cells covers the bottom of the dish. The cover slips are removed with sterile forceps and treated as in the bone marrow method. Again no colchicine is necessary. The cells remaining in the dishes may be subcultured for future examinations. This method is a modification of that described by Harnden [*100*].

Peripheral Blood. Venous blood, 10 to 20 cc., is obtained in sterile vacuum tubes, which have previously been prepared with 0.1 cc. of a 5,000 units per cc. solution of heparin (Liquaemin®). Within two hours, 0.25 cc. phytohemagglutinin (Difco) is added to each 10 cc. of blood. After standing for two minutes, the blood is centrifuged at 200 r.p.m. for five minutes. The serum, containing the white blood cells, is pipetted off, and 2 cc. aliquots are placed in wide 35 cc. centrifuge tubes. Medium 199 is added so as to make the final cell concentration 1 to 3 million per cc. (usually 8 cc. of medium 199 to 2 cc. of cell suspension). The tubes are closed and placed in a 39°C. water bath for seventy-two hours. At this time, colchicine (final concentration 3 gamma per cc.) is added without removing the tubes from the bath. All steps until this point are made with sterile technic. Colchicine is not necessary, but helps to obtain greater numbers of countable mitoses. After two hours the tubes are emptied into conical 15 cc. centrifuge tubes, which are centrifuged at 400 r.p.m. for five minutes. The supernatant is pipetted off, 2 cc. of 1 per cent sodium citrate is added to each tube and the cells suspended. After three minutes the tubes are centrifuged at 200 r.p.m. for four minutes, and the supernatant is pipetted off. A 3:1 mixture of absolute ethyl alcohol and glacial acetic acid is added slowly, drop by drop, resuspending after each drop, until 2 cc. have been added. After ten minutes the suspension is centrifuged at 200 r.p.m. for four minutes, and the supernatant is pipetted off. The cells are now suspended in 2 cc. of 45 per cent acetic acid, where they are permitted to settle for twelve hours or more in the refrigerator. The supernatant is pipetted off, and the cells are resuspended in 1 to 2 cc. of the alcohol-acetic acid mixture. Several small drops are distributed on a clean cover slip and permitted to dry. Temporary preparations may be made by placing a drop of acetic orcein (as already described) on a slide, inverting the cover slip on the drop, and removing the excess stain by placing filter paper along the edges of the cover slip. This preparation is sealed with Kronig's cement. Permanent preparations are made by placing the unstained cover slip in a staining rack, which is taken through the following solutions: acetic orcein, twenty-five minutes; tertiary butyl alcohol, one minute; tertiary butyl alcohol, one minute; tertiary butyl alcohol, two minutes; 1:1 tertiary butyl alcohol/xylene, two minutes; xylene, six minutes; xylene, two minutes. While still wet with xylene, the cover slip is inverted on a slide into a drop of balsam or Permount. This method is a modification of those described by Miller [*101*] and Hungerford et al. [*87*].*

All types of preparations are then studied by microscopic examination under oil immersion, photomicrography, and by cutting out individual chromosomes from enlargements for final matching.

ADDENDUM

Since this paper was submitted for publication, many articles have appeared in the literature which describe material of importance to human cytogenetics. We have selected a group of these references for discussion. No attempt has been made to be comprehensive. The works cited are ones which seem to have general importance.

Sex Chromosomes. DeGrouchy et al. [*107*] have described a case of gonadal dysgenesis with female phenotype but XY sex chromosome constitution. This is similar to Harnden's case [*92*], but laparotomy disclosed one small primitive gonad.

Stewart and Sanderson [*108*] and Fraser et al. [*109*] have described further XXX females, and have demonstrated that some of these individuals have unimpaired fertility. High correlation with mental deficiency is suggested, but incidence in the general population is unknown.

Carr et al. [*110*] have described two mentally defective patients with XXXX sex chromosome constitutions. This again demonstrates the relationship of the "sex chromatin" to X chromosomes: three chromatin masses were found in many buccal mucosal cells presumably carrying four X chromosomes, i.e., one less chromatin mass than number of X chromosomes.

Barr et al. [*111*] and Miller and Breg [*112*] have described mentally defective patients apparently exhibiting Klinefelter's syndrome who had sex chromosome constitutions of XXXY [*111*] and XXXXY [*112*]. This demonstrates the masculinizing effect of the Y chromosome even in the presence of multiple X chromosomes.

Muldal et al. [*113*] have described still another patient with Klinefelter's syndrome, the "double male," in whom the sex chromosome constitution was XXYY.

Mosaicism. Cooper et al. [*114*] have described another type of sex chromosome mosaicism in which peripheral blood leukocytes had XYY sex chromosome constitution, while cells grown

* This method has been further modified, as mentioned in the Addendum.

from skin biopsy specimens were primarily XO. Clinically the patient was a female with gonadal dysgenesis.

That autosomal mosaicism does occur was shown by Clarke et al. [*115*] in a patient with mongolism, in whom some cells had trisomy for chromosome 21, while the remainder were normal.

Translocation. Carter et al. [*116*] and Penrose et al. [*117*] have reported on patients with mongolism who had a translocation with consequent joining of two acrocentric chromosomes into a "new" metacentric. Together with reference 81, these papers demonstrate the existence of a class of mongols who have a triple dose of chromosome 21, but only forty-six chromosomes. This is due to the translocation and fusion of two chromosomes into one. They also show that normal parents and relatives of these mongols frequently have only forty-five chromosomes including the translocation. These parents have a high expectancy of producing mongoloid children because of the abnormal translocation chromosome they carry.

Another report, by Moorhead et al. [*118*], also describes familial transmission of translocated chromosomes. It also suggests an increased risk of meiotic non-disjunction in subjects carrying such translocations. This indicates the importance of studying normal relatives of patients with chromosomal anomalies.

Radiation and Leukemia. The effect of heavy doses of therapeutic radiation on chromosomes was described by Tough et al. [*119*]. In a group of patients with ankylosing spondylitis treated by radiation, multiple non-specific chromosomal anomalies were found in peripheral blood leukocytes up to four weeks after exposure to radiation.

Nowell and Hungerford [*120,121*], Baikie et al. [*122*] and Tough et al. [*123*] have made an important contribution by demonstrating a specific chromosomal anomaly related to chronic myelocytic leukemia. A minute chromosome is found in place of either a number 21 or 22 in the leukemic cells.

Autosomal Trisomy. A report by Hayward and Bower [*124*] suggests trisomy of number 22 in Sturge-Weber syndrome, but the finding is not supported by further work of the same and other authors.

Satellite Abnormalities. Tjio et al. [*125*] have described enlarged satellites on one of the acrocentric chromosomes in two patients with Marfan's syndrome. However, Cooper and Hirschhorn [*126*] have described a family in which a short acrocentric chromosome with enlarged satellites is transmitted from a normal father to three of his four children. Two of the children with the enlarged satellites are normal, while the third (the propositus) has some features of mongolism and central nervous system defects. Hirschhorn et al. [*127*] have described another family in which the propositus was a mongol with acute leukemia who also had a translocation between 21 and 22, and enlarged satellites on a long acrocentric. Here also the chromosome with enlarged satellites was transmitted from a normal father to three of four children; only the propositus was abnormal, the other two children with enlarged satellites being normal. The fact that the chromosome bearing the enlarged satellites may be followed through several generations suggests its use as a marked chromosome for genetic linkage studies.

German et al. [*128*] have also described leukemia in a mongol with a translocation. There were no enlarged satellites in this case.

Methodology. Harnden [*129*] has reviewed technics of chromosome examination. In this paper mention is made of the fact that satellited chromosomes tend to associate with one another by their satellited ends. This finding was confirmed thereafter by Ferguson-Smith and Handmaker [*105*].

Hastings et al. [*130*] have described a modification of the technic of peripheral blood leukocyte culture. Their method involves separation of polymorphonuclear leukocytes by phagocytosis of iron particles and their removal by magnetization, giving reproducible results.

Clinicopathologic Conference

Hypertension, Oliguria, Arthralgias, Abnormal Esophageal Motility and Glomerulonephritis

STENOGRAPHIC reports, edited by Lillian Recant, M.D. and W. Stanley Hartroft, M.D. of weekly clinicopathologic conferences held in the Barnes and Wohl Hospitals, are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Internal Medicine and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

A FIFTY-ONE year old white man (G. N.) was transferred to the Barnes Hospital from Hannibal, Missouri, because of oliguria and increasing mental confusion.

The patient had been in good health until the summer of 1959 when he was found to have tachycardia and an elevated blood pressure. He was given digitalis but because of nausea this was discontinued. In November 1959 aching developed in his hips, fingers, wrists and back. He was given a cortisone preparation but it was discontinued because of fluid retention. The patient was hospitalized in Memphis, Tennessee, in February 1960 with a low grade fever and weight loss. He was also found to have leukoplakia of the vocal cords. There was a purplish swelling of the hands and fingers, and tenderness in the hands, right ankle and both knees. At this time narrowing of the fifth cervical intervertebral disc was found, and an electrocardiogram revealed sinus tachycardia. While in the hospital the patient had an abscessed tooth extracted. After discharge from the hospital he was given a three-week course of Lugol's solution as a therapeutic test, but there was no change in heart rate.

The patient was re-admitted to the hospital in Memphis in May 1960. At this time he complained of dizzy spells, severe headaches, weakness and anxiety. Physical examination revealed a blood pressure of 190/126 mm. Hg and signs of congestive heart failure. Pigmentation was present over the face, neck, upper extremities and upper portion of the trunk. An electrocardiogram revealed a sinus tachycardia of 136 per minute. Chest roentgenogram revealed the heart size to be at the upper limits of normal. The patient was given digitalis, Hydrodiuril® and a low salt diet. The blood pressure became normal with the patient on a regimen of 1 mg. Serpasil® daily. A skin biopsy was performed which was non-diagnostic. A Congo red test showed 58 per cent dye remaining at the end of one hour. Serum proteins were normal and lupus erythematosus preparations were negative. Serum iron was 56 mg. per cent and serum iron-binding capacity was 123 mg. per cent. Blood count was normal except for a mild anemia. Urinalysis revealed a specific gravity of 1.022, trace of acetone, 2-plus protein, 8 to 10 white blood cells, 4 to 6 red blood cells, and an occasional granular cast per high power field. Protein-bound iodine, alkaline phosphatase and serum calcium were normal. Serum phosphorus was 5.2 mg. per cent. Serum sodium, potassium and chloride were normal. Urinary 17-ketosteroids and 17-hydroxycorticoids were normal. On admission blood urea nitrogen was 56 mg. per cent and on discharge 75 mg. per cent. An upper gastrointestinal series revealed an irritable and deformed duodenal bulb. A glucose tolerance test revealed a diabetic-type curve.

The patient was transferred to the Mayo Clinic on May 21 for further evaluation. On physical examination there was increased pigmentation of the skin and a blood pressure of 180/110 mm. Hg. Urinalysis revealed an occasional granular cast and red and white blood cells. The hemoglobin value was 11.2 gm. per cent, the white blood cell count was 11,300/cu. mm., and the differential showed 16.5 per cent lymphocytes, 13 per cent monocytes, 65 per cent neutrophils, 3.5 per cent eosinophils, 1.5 per

cent basophils and 0.5 per cent early myelocytes. The blood urea nitrogen was 119 mg. per cent, serum sodium 140 mEq./L., potassium 4.9 mEq./L., chloride 87.1 mEq./L. The serum bilirubin was normal. The sedimentation rate was 112 mm. in one hour (Westergren method). The prothrombin time was nineteen seconds, coagulation time nine minutes, bleeding time three and a half minutes. Results of cephalin cholesterol flocculation, thymol turbidity and bromsulphalein liver function tests were normal. Lupus erythematosus preparations were negative. Serum protein electrophoresis showed 3.37 gm. per cent albumin, 0.33 gm. per cent alpha$_1$-globulin, 0.63 gm. per cent alpha$_2$-globulin, 0.87 gm. per cent beta globulin, and 1.08 gm. per cent gamma globulin. Urine urophorphyrin and porphobilinogen were negative. The urine was tested for catecholamines with negative results. No cryoglobulins were present in the blood. Electrocardiographic studies failed to show evidence of abnormality. Esophageal motility studies revealed findings compatible with scleroderma. The patient was discharged from the Mayo Clinic and was re-admitted after a few days for a renal biopsy; the specimen was reported as showing glomerulonephritis.

On the way home after discharge from the Mayo Clinic, the patient had a grand mal convulsion. He was admitted to a hospital in Hannibal, Missouri, on June 6, 1960. Laboratory studies at this time revealed a hematocrit of 40 per cent and a white blood cell count of 24,500/cu. mm. Serum sodium was 114 mEq./L., potassium 5.1 mEq./L., chloride 76 mEq./L., carbon dioxide 14 mEq./L., non-protein nitrogen 132 mg. per cent, blood sugar 267 mg. per cent. The patient was treated with Cedilanid,® insulin, hypertonic saline solution and ⅙ molar lactate solution. He was found to be weak, confused and lethargic. He was transferred to the Barnes Hospital for further therapy.

In the past the patient had undergone studies which indicated a sensitivity to spring grasses, dust and ragweed.

On physical examination the temperature was 37.6°c., pulse 160 per minute, blood pressure 220/110 mm. Hg, and respirations 24 per minute. The pupils were small and unreactive to light. Fundi revealed arterial narrowing, arteriovenous nicking, hemorrhages, exudates and blurring of the disc margins. There were a few fine rales at the base of the left lung. The heart was enlarged to the anterior axillary line and the apical impulse was diffuse. The rhythm was regular. A diastolic gallop rhythm was present, as was a systolic third heart sound. The liver was palpated 6 cm. below the right costal margin and was tender. There was 4-plus presacral edema and a trace of pretibial edema. The patient was lethargic and disoriented. Generalized muscular weakness was present. The deep tendon reflexes were hyperactive. There was a yellowish brown pigmentation over the exposed areas of the skin.

Laboratory data were as follows: The hemoglobin value was 13.9 gm. per cent; white blood cell count 24,500/cu. mm.; the differential showed 2 per cent stab forms, 91 per cent segmented neutrophils, 5 per cent lymphocytes, 2 per cent monocytes. The urinalysis showed 4-plus albumin, sugar negative, 6 to 8 red blood cells and a few hyaline and granular casts per high power field. The stool guaiac test result was positive. Blood urea nitrogen was 125 mg. per cent, serum sodium 130 mEq./L., potassium 4.7 mEq./L., chloride 84 mEq./L., carbon dioxide 20.8 mEq./L. A lupus erythematosus preparation was negative. The antistreptolysin O titer was 25 units; a urine culture on June 10 revealed a heavy growth of Escherichia coli; a urine culture on June 11 revealed no growth. A sputum culture revealed a few alpha hemolytic streptococci, a few neisseria, two colonies of yellow hemolytic staphylococci and a moderate growth of E. coli; a stool culture revealed no pathogens. Four blood cultures revealed no growth.

An electrocardiogram taken on the day of admission was interpreted as showing probable paroxysmal atrial tachycardia. The venous pressure was 100 mm. saline. Chest roentgenograms taken on admission revealed cardiac enlargement and pulmonary congestion. A roentgenographic examination of the abdomen revealed bilateral small kidneys and sclerosis of the abdominal aorta. Roentgenographic examination of the skull was within normal limits. Dilantin® therapy was started on admission.

Laboratory data on June 9 revealed a blood urea nitrogen of 148 mg. per cent, serum creatinine 12.6 mg. per cent, sodium 133 mEq./L., potassium 6.1 mEq./L., chloride 89 mEq./L., carbon dioxide 20.7 mEq./L., fasting blood sugar 120 mg. per cent, uric acid 12.7 mg. per cent, serum calcium 8.7 mg. per cent, phosphorus 10.7 mg. per cent, serum albumin 3.6 gm. per cent, serum globulin 3.1 gm. per cent,

serum glutamic oxaloacetic transaminase 49 units, serum glutamic pyruvic transaminase 15 units. A lumbar puncture was performed on the same day, with an initial pressure of 200 mm. water and a closing pressure of 150 mm. water. The cell count revealed only 3 red blood cells; the spinal fluid protein was 50 mg. per cent, chloride 116 mEq./L., sugar 103 mg. per cent. An India ink preparation was negative. Colloidal gold curve was normal. Wassermann reaction was negative. Spinal fluid culture revealed no growth on thioglycollate broth or blood agar plate. McCleod's medium was overgrown with fungus. Spinal fluid for culture revealed no growth. A repeat electrocardiogram revealed left ventricular enlargement, and either rapid sinus tachycardia or supraventricular tachycardia and digitalis effect.

On June 10 blood was obtained for culture and then therapy with penicillin, streptomycin, and Achromycin® was started. On the day prior to death, laboratory data revealed the blood urea nitrogen to be 194 mg. per cent, serum sodium 141 mEq./L., chloride 92 mEq./L., potassium 4.6 mEq./L., carbon dioxide 21.1 mEq./L., blood creatinine 15.4 mg. per cent, urine creatinine 66 mg. per cent, hemoglobin 8.1 gm. per cent, white blood cell count 30,800/cu. mm. with 3 per cent metamyelocytes, 6 per cent band forms, 80 per cent segmented neutrophils and 11 per cent lymphocytes. On the morning of death the patient passed several black tarry stools. Blood replacement was given at this time. He received 2 units of packed red blood cells and 3 units of whole blood. At one point his blood pressure fell to 50/30 mm. Hg and subsequently returned to 110/80 mm. Hg. However, about four hours later he became cyanotic, weak and labored respirations developed and he died.

During the hospital course the patient had fever with temperatures varying between 38°–39°C. The temperature fell to normal on the day before death. The blood pressure varied between 190–210/110–140 mm. Hg until June 11 when it decreased to 160/90–100 mm. Hg. The intramuscular administration of Serpasil® was started on June 10. The patient remained oliguric during his hospital course and his fluid intake was limited.

CLINICAL DISCUSSION

DR. EDWARD H. REINHARD: The patient under discussion presented a complex problem with manifestations of hypertension, oliguria, renal failure, abnormal esophageal motility, arthralgias and a renal biopsy report of glomerulonephritis. Dr. Brown, would you comment on the significance of the low serum iron in this patient?

DR. ELMER BROWN: I assume that the serum iron was determined because of the question of hemochromatosis in view of the presence of skin pigmentation and diabetes. This low serum iron rules out that diagnosis. When the serum iron is low and the unsaturated iron-binding capacity is also low, the pattern is one seen in chronic renal disease, infection, malignancy and a whole variety of chronic diseases.

DR. REINHARD: Dr. McAlister, will you comment on the possibility of this patient having had scleroderma. Since we do not have the esophageal films, I will ask you to comment on the following: How specific are the changes on fluoroscopic examination of the esophagus for scleroderma? Are the changes in motility similar to those seen in other collagen diseases, or are the changes in scleroderma fairly specific?

DR. WILLIAM H. MCALISTER: The changes are not specific. Dermatomyositis, in particular, will have indistinguishable findings. Somewhat similar changes will be found in some elderly people.

DR. REINHARD: Would you now discuss the roentgenograms taken at the Barnes Hospital.

DR. MCALISTER: On plain roentgenograms of the abdomen, the bony structures appeared normal. Some calcifications were present in the spleen and aorta. The liver appeared to be slightly enlarged. The renal outlines were small, but without evidence of calcification. The spine showed some degenerative changes. The chest roentgenograms taken on admission showed cardiomegaly and pulmonary congestion. A small calcified granuloma was present in the upper lobe of the left lung. An infiltrate in the lower lobe of the right lung with decreased volume could be seen. A chest roentgenogram taken two days later showed significant increase in cardiac size, increased pulmonary congestion and pulmonary edema. Superimposed pneumonic disease cannot be excluded.

DR. REINHARD: From a clinical standpoint it is important to decide which came first, the hypertension or the renal disease. The first impression that anyone would get from reading this case summary, and probably the correct impression, is that the hypertension developed secondary to severe renal disease. However, we must at least consider the possibility that this

patient had malignant hypertension as a primary disease. Dr. Massie, would you comment on this?

DR. EDWARD MASSIE: Yes, one must consider the possibility that the patient had severe hypertension with secondary renal disease. He satisfies many criteria for malignant hypertension. His eye grounds are typical with exudate, hemorrhages and blurring of disc margins. He has a blood pressure that at times rose above a diastolic level of 130. He had a fairly rapid downhill course. Many of these patients die within a year, and renal function will often deteriorate within six months.

DR. REINHARD: This man had azotemia when first seen which was very shortly after the onset of symptoms. Would this be somewhat disturbing?

DR. MASSIE: This is disturbing, and if it were not for that consideration, I would say that he was in the malignant phase of hypertension. With that point in mind, I would not make this diagnosis. I am reminded of an unpublished study made about twenty or more years ago at the Peter Bent Brigham Hospital. One of the residents studied the level of blood pressure in patients with malignant hypertension in contrast to the level in patients with primary renal disease and secondary hypertension. If one were to make a deduction from that particular study alone, one would say this patient had primary hypertension with secondary renal disease.

DR. REINHARD: We will eventually touch upon some other points that make the diagnosis of malignant hypertension as the primary disease in this patient unlikely. I would like to consider first the type of renal disease that might be involved here. One of the very early manifestations of the disease in this patient was swollen, painful joints associated with low grade fever. Among other conditions, rheumatoid arthritis was considered as a possible explanation for these joint manifestations. Rheumatoid arthritis is certainly a disease that involves various viscera in addition to the joints. The existence of specific renal lesions in association with rheumatoid arthritis have also been suggested.

Dr. Morrin, could you tell us briefly about the renal lesion of rheumatoid arthritis, if there is one?

DR. PETER A. F. MORRIN: Although glomerulitis has been described in some patients with chronic rheumatoid arthritis, severe azotemia and renal failure did not develop in these cases. Also, a recent biopsy series* did not reveal any specific glomerular lesion in thirty cases of rheumatoid arthritis.

DR. REINHARD: Dr. Brown, amyloidosis was apparently considered as a possible explanation for the visceral involvement in this case. When the patient was first seen in Memphis, a Congo red test was performed. Do you think we should give this disease serious consideration?

DR. BROWN: Since the Congo red test showed a retention of 58 per cent at the end of an hour, I think it is reasonably good evidence that there was no amyloid deposition of any great degree. It is said that less than 10 per cent retention in the blood is a diagnostic test. This patient has the added effect of the Congo red dye leaving in the urine; so that even with this loss, he does not come anywhere close to the diagnostic blood level.

DR. REINHARD: The presence of marked proteinuria may make it difficult to interpret the Congo red test; but proteinuria tends to give a false positive rather than a false negative result. Furthermore, the kidneys were very clearly seen on the roentgenograms, and they were small. This also would be against amyloid deposition.

Now let us again consider the diagnosis of scleroderma. This diagnosis was mentioned and discussed by various people who saw the patient. The skin was described by the doctors at the clinic in another city as being somewhat thickened with loss of elasticity. This was not emphasized in the chart here at Barnes Hospital. There was increased pigmentation on the upper part of the body. Esophageal motility studies were described as being abnormal and "compatible with scleroderma." Scleroderma is certainly a diffuse disease, and involves many organs including not only the gastrointestinal tract, but also the kidneys. Brownish pigmentation of the skin is seen very commonly in scleroderma. Dr. Morrin, do you think that this patient might have had sclerodermatous renal disease?

DR. MORRIN: Scleroderma will explain the whole course here, Dr. Reinhard, except for the renal biopsy. Glomerulonephritis is not a characteristic finding in scleroderma. The changes

* POLLOK, V. E., PIRANI, C. L., MUEHRCKE, R. C., PULOS, P. C., KARK, R. H. and STECK, I. On renal involvement in systemic lupus erythematosus and other so-called collagen diseases.

that are found may be indistinguishable from malignant hypertension. It is a disease which involves the intralobular arteries and may extend up into the arterioles with occasional necrosis of the glomeruli. Sometimes there will be increased cellularity of the glomeruli, and thickening of the basement membrane has been reported. An inexperienced pathologist might conceivably mistake this for glomerulonephritis, but I do not think this is likely.

DR. REINHARD: One thing which to me seemed to be very much against scleroderma in this case is that when renal involvement and severe renal failure does occur in scleroderma, it almost always occurs as a very late manifestation, and almost invariably in patients with obvious scleroderma elsewhere, including the skin. Is that not correct?

DR. MORRIN: Not absolutely. It is generally believed that when patients have renal involvement with scleroderma, they run a rapidly progressive downhill course. This is true when the renal failure becomes clinically apparent. However, in a series of thirty-one autopsy cases reported by Piper and Helwig,* thirty patients had either clinical or pathologic evidence of renal involvement, but only five of these died of uremia.

DR. REINHARD: Yes, but of those patients who died of uremia, by the time they died did they not have fairly obvious sclerodermatous involvement of the skin?

DR. MORRIN: Yes, this is true.

DR. REINHARD: It seems to me that if this patient had scleroderma he never had significant skin involvement; this would make severe renal failure primarily on this basis unlikely.

Let us consider other types of collagen disease. I believe that we can rule out scleroderma with a fair degree of confidence here, but we certainly cannot rule out certain other collagen diseases with any degree of certainty. Dr. Morrin, do you think that the renal lesion in this case might have been due to polyarteritis or to lupus erythematosus? In the renal failure associated with these two diseases, can the pathologic picture based on a small needle biopsy specimen simulate glomerulonephritis? Would this be more easy to mistake for glomerulonephritis than in the case of scleroderma?

DR. MORRIN: Yes. A periarteritis could certainly fulfill all the criteria. One would like to see more visceral symptomatology than this patient demonstrated, but I do not think you can rule out periarteritis. The patient had tachycardia, joint manifestations, low grade fever and renal involvement with hypertension. He also belonged to the right sex and age group. There is one point that might be against this diagnosis. In periarteritis it is believed that the renal disease precedes the hypertension. However, we do not have enough evidence to be sure which came first in this case. With regard to lupus erythematosus, the patient had small kidneys; that is not characteristic of this disease. In addition, no evidence of lupus was found in the biopsy specimen, and in some instances a renal biopsy can be definitely diagnostic. Four lupus erythematosus preparations were negative. The sex and age were atypical, and there was no evidence of serositis, skin rash or lymphadenopathy which are frequently present in lupus erythematosus. I do not think we can completely exclude this diagnosis, but I think it is very unlikely.

DR. REINHARD: If a patient did have far advanced renal disease due to periarteritis, do you think that on needle biopsy of the kidney it might produce changes which would be more readily confused with glomerulonephritis than in the case of scleroderma?

DR. MORRIN: Yes. However, there are some borderline cases in which the picture is indeterminate between lupus erythematosus, periarteritis and scleroderma. This patient may well have manifested features of more than one of these diseases.

DR. REINHARD: Dr. Sherry, what do you think about the possibility of either lupus erythematosus or periarteritis in this case?

DR. SOL SHERRY: If this patient had a connective tissue disorder, which is a likely possibility, then periarteritis would be my first choice. I was impressed by the fact that most of the clinical findings could be explained on the basis of vascular disease, a predominant manifestation in periarteritis, and that there was early renal involvement, moderately severe hypertension and a fairly good allergic history. Such findings seem to fit better with periarteritis than lupus erythematosus, in which connective tissue manifestations and immune type of phenomona usually predominate to a greater extent.

DR. REINHARD: There were also four negative lupus erythematosus preparations, made in

* PIPER, W. N. and HELWIG, E. B. Progressive systemic sclerosis. Visceral manifestations in generalized scleroderma. *Arch. Dermat. & Syph.*, 72: 535, 1955.

three different hospitals, and certainly the rather marked leukocytosis would fit in a little bit better with periarteritis than with disseminated lupus. We certainly have to consider one final possibility, and that is that the patient had what the needle biopsy specimen is said to have shown, acute glomerulonephritis. Dr. Morrin, what do you think about this possibility?

DR. MORRIN: I think we would find it hard to explain the joint manifestations and the persistent tachycardia. The prolonged fever would also be unusual. Most of the other findings are compatible with glomerulonephritis.

DR. SHERRY: I was intrigued by the observation that the urinary specific gravity was noted to be 1.022 even though the patient was quite azotemic. If a laboratory error can be excluded, would not such a concentrating power be incompatible with a glomerulonephritis severe enough to produce significant azotemia? I wondered whether this finding is more in favor of involvement of large or medium-sized renal arteries sufficient to produce a fairly extensive reduction in blood flow, but with glomerulotubular function fairly well maintained; i.e., a sort of intrarenal prerenal azotemia.

DR. MORRIN: The value of 1.022 is perhaps a little higher than one would expect to see, but it is possible to get a concentrated urine in glomerulonephritis. The usual explanation postulates that glomerular filtration is decreased relatively more than tubular function and that functioning tubules acting on a decreased volume of glomerular filtrate can concentrate the urine more than in other forms of chronic Bright's disease such as pyelonephritis. Even with azotemia, one occasionally may find a concentrated urine.

DR. REINHARD: I believe that periarteritis would best explain the findings in this case, provided the patient has only one disease and that all the manifestations that have been discussed are attributable to one disease. On the other hand, if this patient had several diseases, then I believe that we can best explain the sequence of events in this case on the basis of some type of acute glomerulonephritis, plus some type of unrelated arthritis, such as rheumatoid arthritis. I personally would be willing to dismiss the diagnosis of malignant hypertension, because of the points that have already been mentioned, plus the fact that a needle biopsy specimen of the kidney did show glomerulonephritis, or some lesion that closely simulated glomerulonephritis.

PATHOLOGIC FINDINGS

DR. W. STANLEY HARTROFT: The clinical discussion indicates problems on which to focus attention at autopsy. Consideration will be given questions of collagen disease with arterial lesions, of glomerulonephritis and of hypertension.

My samples of skin came from thoracic and abdominal areas in which sclerosis was evident throughout subcutaneous layers. (Fig. 1.) Dermis in these regions was thinned. However, not only were vessels normal, but also and significantly, skin appendages were not completely absent (Fig. 2), although they were decreased relative to normal. In striated muscle a few fibers were swollen but they were unaccompanied by inflammatory reaction.

Abnormalities of brain and spinal cord were limited to small petechiae in pons with recent softening, but cerebral vessels in microsections were normal.

Atrophy of esophageal muscularis with fibrosis and associated deposits of fibrin were prominently evident throughout. (Fig. 3.) Longitudinal muscle coats were well preserved except for edema, although circular layers were atrophic. (Fig. 4.) Focal collections of rather large numbers of lymphocytes beneath epithelium were mingled with surprising numbers of plasma cells, but vessels were normal.

Three chronic ulcers were found in the duodenum. Ecchymoses involved the stomach and remainder of the small intestine; the colon was quite edematous. Despite ascites, the mesenteric vessels were normal.

The liver was congested secondary to terminal myocardial failure. Periportal fat had accumulated to minor degree. Connective tissue of Glisson's capsule with its triadal infoldings was normal in amount and the vessels were normal.

The spleen was extensively congested, again reflecting myocardial failure, but small splenic vessels were no thicker than usual.

Throughout the lungs, abnormal fibrin deposits not only lined many alveolar septuma (Fig. 5) but also often completely filled the air spaces—a picture of extensive fibrinous pneumonia (Fig. 6)—although the term "pneumonia" is not happily used in this sense, usage condones it. Fibrin deposits were so extensive that pulmonary collapse on opening the chest was prevented. Some alveolar walls were truly fibrotic—often extensively so. (Fig. 7.) This

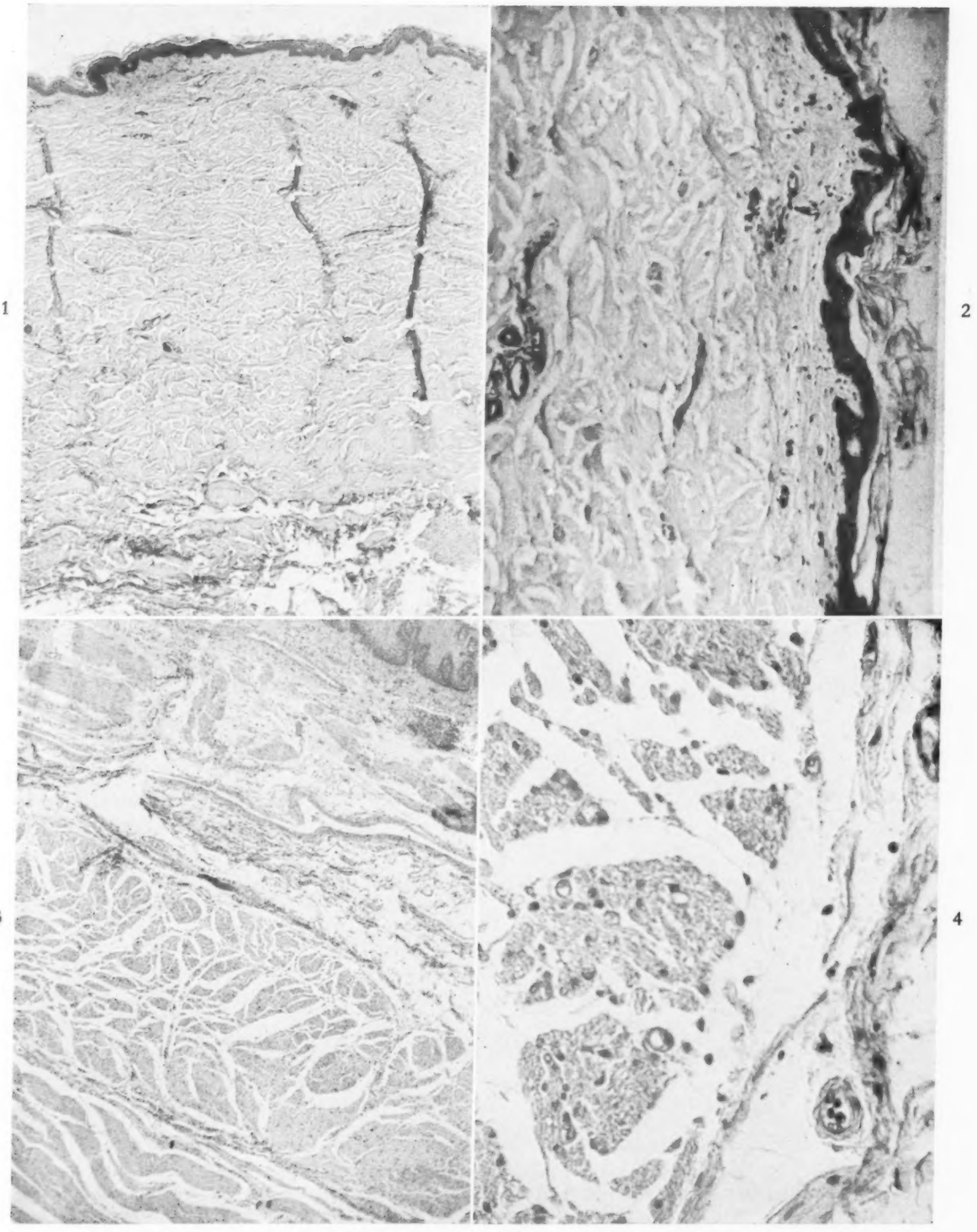

FIG. 1. In this section of skin from the anterior wall of the thorax, subdermal sclerosis is evident. The overlying dermis is thin. Paraffin section; hematoxylin and eosin stain, magnification × 30.

FIG. 2. Higher magnification of thoracic skin specimen reveals a few vestigial skin appendages despite the advanced degree of sclerosis. Paraffin section; hematoxylin and eosin stain, magnification × 100.

FIG. 3. The epithelium lining the esophagus is included in the upper right hand corner. Beneath it there is extensive edema and fibrinous deposition throughout the submucosal layer. The fasciculi of circular and longitudinal muscle coats are separated by edema. Paraffin section; hematoxylin and eosin stain, magnification × 30.

FIG. 4. In this view of the esophagus wall, atrophy of the fasciculi of the smooth muscle bundles is apparent as well as their wide separation by edema. The muscle bundles consist of granular atrophic cytoplasm surrounding the dark pyknotic nuclei. A portion of longitudinal muscle (right) is not as severely affected. Small arteries and arterioles are normal. Paraffin section; hematoxylin and eosin stain, magnification × 200.

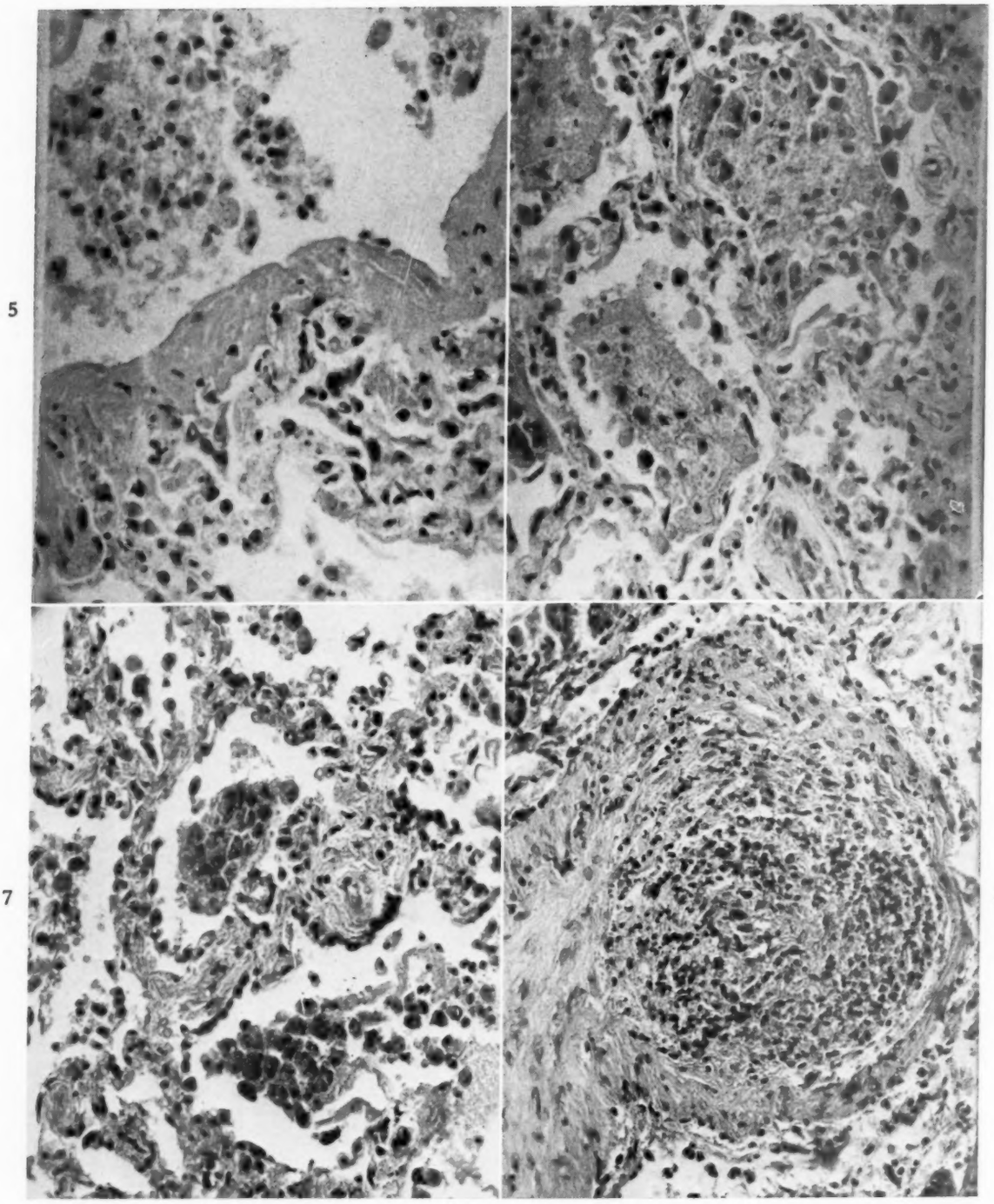

FIG. 5. The field includes a portion of an alveolar space and the wall of an alveolar sac. The wall of the alveolar sac is lined by a very thick layer of eosinophilic, homogenous, almost anuclear material, composing together a hyaline membrane. Paraffin section; hematoxylin and eosin stain, magnification × 300.

FIG. 6. In this area of the lung two alveolar spaces are completely filled with granular strands of fibrin in which are intermixed leukocytes of various kinds. The alveolar septums are thickened by congestion of their capillaries. Paraffin section; hematoxylin and eosin stain, magnification × 250.

FIG. 7. The alveolar spaces depicted here are filled with hemosiderin-laden macrophages ("heart-failure" cells). The alveolar septums are significantly thickened by fibrous tissue. Thick walls are covered by a continuous layer of epithelium visible even by light microscopy. Paraffin section; hematoxylin and eosin stain, magnification × 250.

FIG. 8. This rather large artery in the pancreas exhibits extensive fibrinoid necrosis and polymorphonuclear infiltration of the inner layers of its walls (intima and subintima) with almost complete occlusion of the lumen. Parenchyma supplied by this vessel is necrotic. Note that the distended media and adventitia are relatively normal. Paraffin section; hematoxylin and eosin stain, magnification × 200.

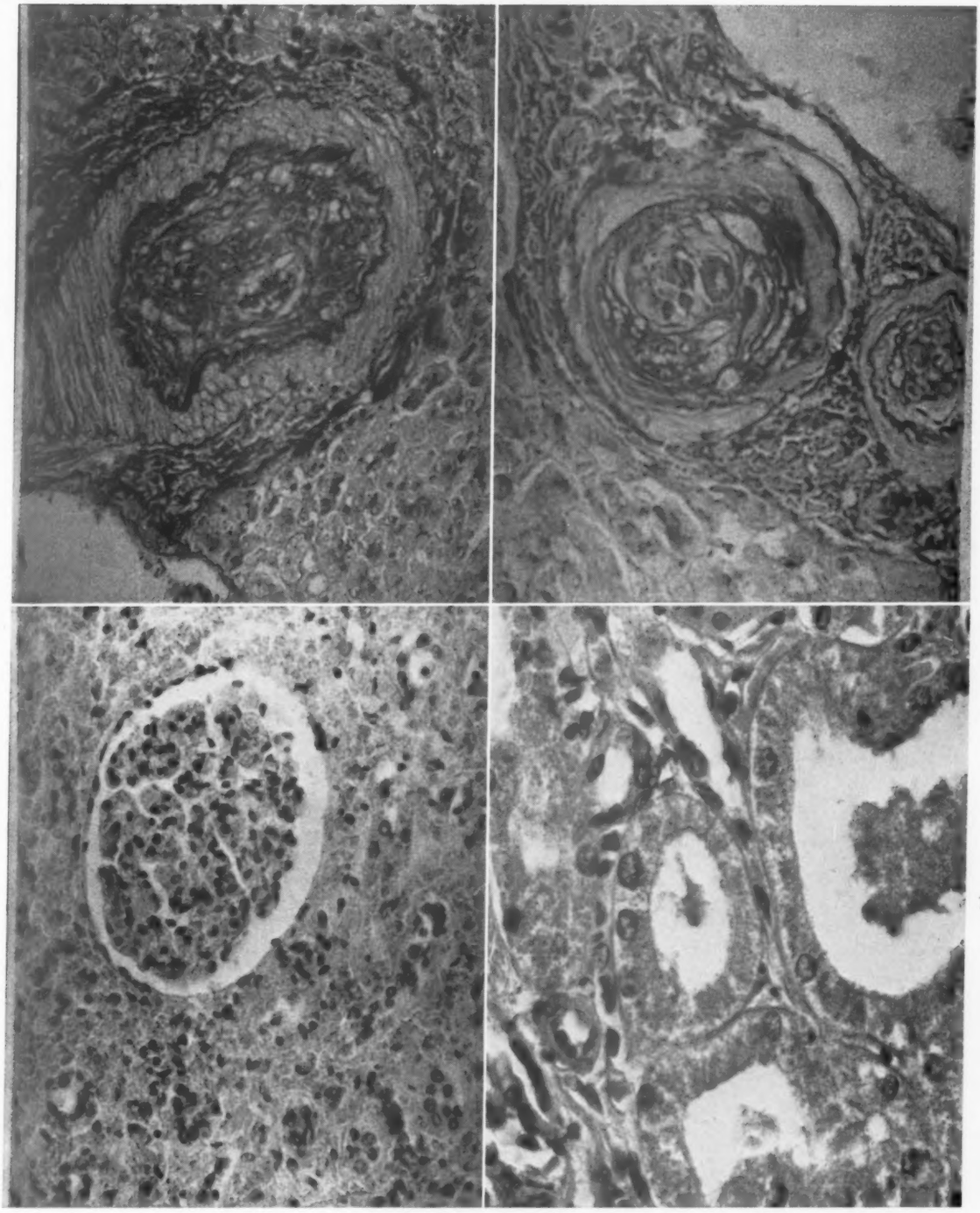

FIG. 9. A chronic lesion has almost obliterated the lumen of this pancreatic artery. The lumen has been greatly reduced by a thick layer of subintimal sclerotic tissue (dark grey in the photograph). All this abnormal deposit of collagen is within the internal elastic laminae which appears as a darker, almost black line. Parenchyma supplied by this vessel was atrophic. Verhoeff-van Gieson stain, magnification × 200.

FIG. 10. The vessel represented is a very small artery (prearteriolar in size). Its lumen has been almost completely obliterated by subendothelial hyperplasia and some sclerosis. The media is not primarily involved. Paraffin section; Verhoeff-van Gieson stain, magnification × 500.

FIG. 11. Aside from some congestion and edema this glomerulus is relatively normal despite the fact that the surrounding tubules are either completely necrotic or almost so. Between the necrotic tubules many red blood cells can be seen as pale grey objects. Paraffin section; hematoxylin and eosin stain, magnification × 300.

FIG. 12. Prominent granular mitochondria occupying almost the entire cytoplasm of the cells forming the walls of these proximal convoluted tubules indicate their hypertrophy. These tubules, presumably overactive, probably represent a form of compensatory hyperplasia for the necrotic tubules elsewhere. Paraffin section; hematoxylin and eosin stain, magnification × 500.

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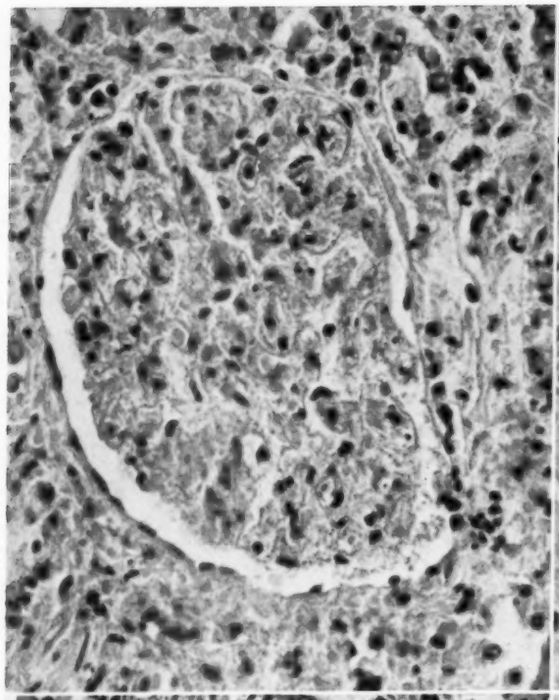

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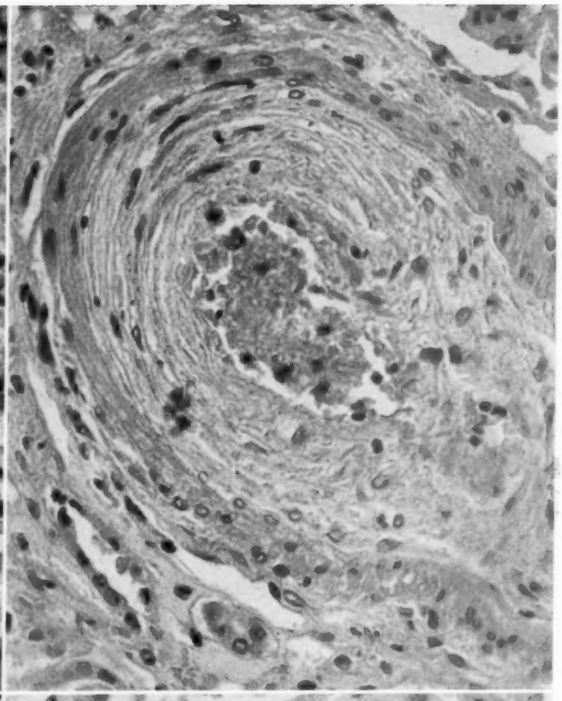

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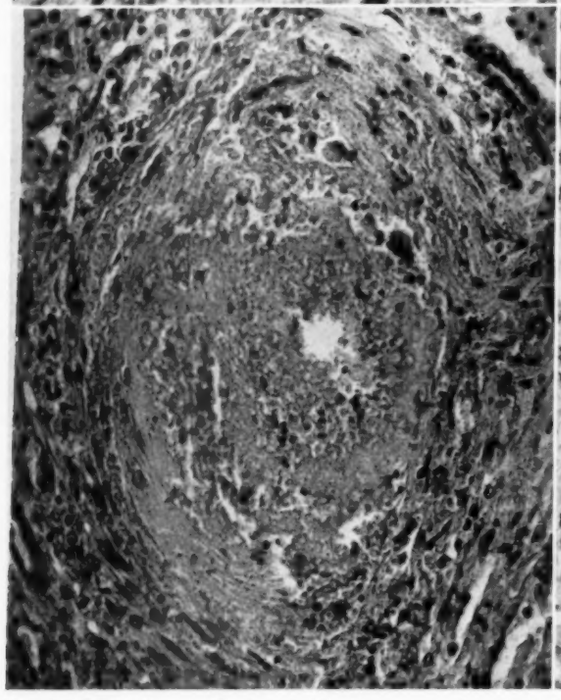

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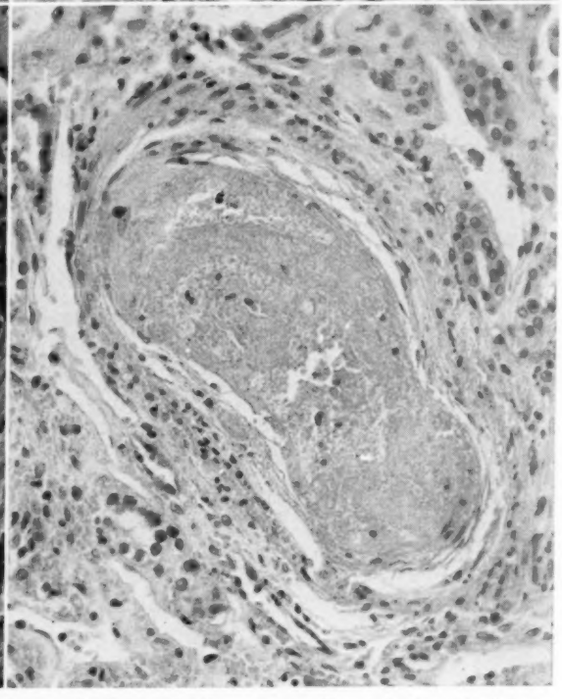

FIG. 13. This glomerulus has undergone an early stage of necrosis along with its surrounding tubules. Nuclei are shrunken and pycnotic while others have lysed. The cytoplasm of these cells has a fibrinoid appearance. Paraffin section; hematoxylin and eosin stain, magnification × 300.

FIG. 14. An interlobular artery near its origin exhibiting extensive subintimal sclerosis occupies the field. Note again that the media (darker grey in photograph) and adventitia are unaffected. Subintimal sclerosis has prevented the vessel from collapsing in its usual way. In the lumen some red blood cells have become entrapped within a recent fibrin thrombus. Paraffin section; hematoxylin and eosin stain, magnification × 300.

FIG. 15. This interlobular artery, also near its origin, is almost completely destroyed by fibrinoid necrosis. Its inner wall, partly destroyed, merges with fibrin thrombus. The greatest destruction again affects the inner layers of the wall; in this case the media has been secondarily involved. The surrounding tissue is necrotic and infiltrated by inflammatory cells. Paraffin section; hematoxylin and eosin stain, magnification × 250.

FIG. 16. A recently organized thrombus, completely obstructs the lumen and distends the walls of this interlobular vessel in the kidney. There is a moderate degree of subintimal sclerosis present. Paraffin section; hematoxylin and eosin stain, magnification × 300.

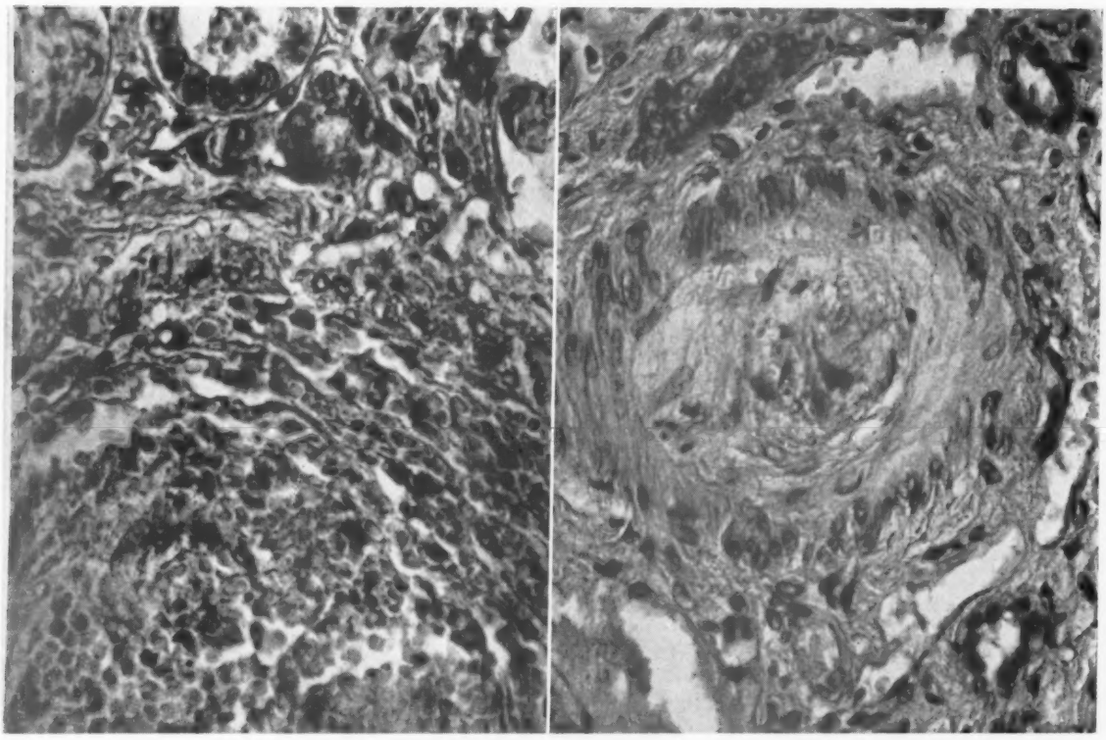

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FIG. 17. A portion of the wall of a interlobular artery is shown near its origin from the arcuate. There is much destruction and necrosis of the inner layers of the wall with red blood cells between them. Paraffin section; hematoxylin and eosin stain, magnification × 400.

FIG. 18. In this small intralobular artery, intimal sclerosis has completely obliterated the lumen of the vessel. Paraffin section; hematoxylin and eosin stain, magnification × 500.

change, along with edema, congestion and pleural effusion was related to myocardial events further reflected by numerous macrophages filled with hemosiderin (heart-failure cells) which crowded alveolar spaces. Pulmonic vessels were normal.

The hypertrophied heart (515 gm.) was remarkably dilated but its vessels in sections was normal.

I am left with the pancreas and kidneys. In the pancreas, subendothelial proliferation strikingly involved many arteries, both small and large. Some, even the largest, underwent fibrinoid necrosis of intimal layers, whereas similar portions in others were marvelously sclerosed. (Fig. 8 and 9.) These lesions, unlike periarteritis, did not involve the media and adventitia. Depending on the nature of the vascular lesion (acute necrosis or chronic sclerotic narrowing) necrosis or atrophy of related parenchyma was prominent. Intimal sclerosis in some small arteries so encroached on their lumens that room for only one red blood cell remained. (Fig. 10.) Islets were affected in hemorrhagic necrotic areas but not in atrophic ones. Little islet reserve remained. If the patient had lived longer, diabetes almost surely would have developed. Small pancreatic arterioles were hypertensively hyalinized.

Rather small kidneys were finely pebbled by hypertensive arteriolar nephrosclerosis. Cortices were riddled by small infarcts of various ages—some young, others old. Both necrotic and atrophic foci were found, the nature of change being dependent on the state of the vessel that supplied them. Recent hemorrhage was seen in many necrotic areas in which glomeruli were better preserved than tubules (Fig. 11), which were often surrounded by polymorphonuclear leukocytes. Tubules in other areas were hypertrophic with overly prominent granular mitochondria. (Fig. 12.)

What about glomeruli? (Biopsy indicated glomerulonephritis.) I was unable to find one to suggest any stage or type of glomerulonephritis. Almost all were pathologic, but these changes

consisted of necrosis, hyalinization, fibrosis, atrophy or hypertrophy. Necrotic glomeruli appeared peculiarly fibrinoid. (Fig. 13.)

The renal vessels were extraordinarily interesting. Lobar and arcuate arteries were normal but truly extensive subendothelial hyperplasia involved most interlobular arteries. (Fig. 14.) Fibrinoid necrosis of others attracted attention to an equal degree. (Fig. 15.) Some of the latter were distended and occluded by recently formed thrombi (Fig. 16), whereas mural hemorrhage of inner layers in other arteries commanded interest. (Fig. 17.) Sclerosis in smaller vessels (intralobulars) sometimes had advanced to the ultimate, obliterating lumens. (Fig. 18.) These lesions were typical of those associated with scleroderma. In addition, renal arterioles (afferent glomerular) were hyalinized—the result of hypertension.

Although in all organs but the pancreas and kidneys, the arteries were normal and even though in subcutaneous tissue and esophagus all the pathologic features associated with scleroderma were not present, I have nevertheless made this diagnosis because the appearances of the kidneys particularly, and of the pancreas were to my mind diagnostic. These lesions were not those of periarteritis, because periarteritis is a panarteritis with degenerative changes (hemorrhage and polymorphonuclear infiltration) involving all coats, not just inner ones. The lesions were not those of lupus erythematosus because in none of the glomeruli did I see "wire-loops"; in neither glomeruli nor lymph nodes could I find hematoxylin bodies; in neither spleen nor lymph nodes did I see typical onion-skin hyperplasia of vessels. Necrosis of lymph nodes was absent.

My *final anatomic diagnoses* are therefore: scleroderma involving skin of thorax and abdomen; concentric subendothelial fibrosis and focal fibrinoid necrosis in small arteries and multiple infarcts in the kidneys; focal atrophy, perifibrillar fibrosis and focal interstitial fibrinous deposition in muscularis of esophagus; arteriolar nephrosclerosis, advanced; hypertrophy of heart (515 gm.) particularly of left ventricle; partly organized fibrinous pneumonia involving upper and lower lobes of right lung (history of uremia); acinar atrophy and focal fibrinous necrosis of pancreatic acini; acute necrosis of small arteries in the pancreas; marked congestion and moderate edema of lungs; mucus in tracheobronchial tree, moderate; chronic duodenal ulcers (three); partly digested blood in stomach, small and large intestines (1,500 ml.); ecchymoses in stomach, jejunum and ileum; chronic passive congestion of liver with slight fatty metamorphosis; acute congestion of spleen; serous pleural effusions (450 ml. on the right, 30 ml. on the left); ascites, 200 ml.; sutured cut-down incision in upper right arm; recent sutured (5 cm.) incision over calf of left leg.

DISCUSSION

DR. MORRIN: Dr. Hartroft, did you have an opportunity to review the renal biopsy specimen?

DR. HARTROFT: No, we did not, although we tried very hard to get it.

Case Reports

Nodular Rheumatoid Lung Disease*

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THE pulmonary manifestations of rheumatoid arthritis have received considerable recognition since Ellman's original reports in 1947 and 1948 [*1,2*]. Although varied and widespread nonspecific findings are being increasingly noted, true rheumatoid nodules situated in the lung or pleura, occurring without known occupational exposure to dust, have remained distinctly uncommon. It is our purpose to report another case of nodular rheumatoid lung disease which was especially interesting because of the association of pulmonary nodules with widespread subcutaneous nodules and a strongly positive reaction to the L.E. test.

CASE REPORT

The patient (H. J.) a fifty-four year old bricklayer, was referred to the hospital in May 1960 because of unusual roentgenographic findings. He had been well until February 1959, when he first noted swelling of the backs of both hands. This was followed by progressive swelling of the hands, wrists, elbows, knees and ankles. His medications during the year prior to being seen here included approximately 2.4 gm. of aspirin and 4 to 8 mg. of 6-methyl-delta-1-hydrocortisone per day. Over the past year he had noted a weight loss of 60 pounds and the progressive development of subcutaneous nodules, both of which had been most rapid over the immediately preceding two months. His chief complaint on admission was weakness, weight loss despite a good appetite, and pain in his knees and upper arms on any attempt at motion. On examination his temperature was 98°F., height 5 feet, 8.5 inches, weight 136 pounds and blood pressure 130/80 mm. Hg. He was emaciated with poor skin color. He could hardly walk because of the pain in his legs. The joint of the left elbow was particularly tender and painful on motion, the knees much less so, and the shoulders and wrists not at all. There was marked synovial thickening of the wrists, elbows and knees (Fig. 1), with lesser involvement of other joints, and ulnar deviation of the hands and wrists. Rheumatoid nodules were noted over the wrists where they were extremely numerous, varying in size from 0.5 to 1.5 cm., soft, non-painful and non-adherent to the skin. They were also found on the extensor surfaces of the fingers and arms, over the shoulders, scapulas, sacrum, ears, scalp, knees, ankles and feet, and along both anterior and posterior ribs. These were firm, non-tender and somewhat yellowish. The patient was unaware of several subcutaneous nodules which suddenly appeared during hospitalization. A few fine rales were noted at the base of the right lung. Otherwise, the physical examination was within normal limits.

Laboratory tests during his hospital admission in-

FIG. 1. The deformity and thickening of the elbows, with subcutaneous nodule formation.

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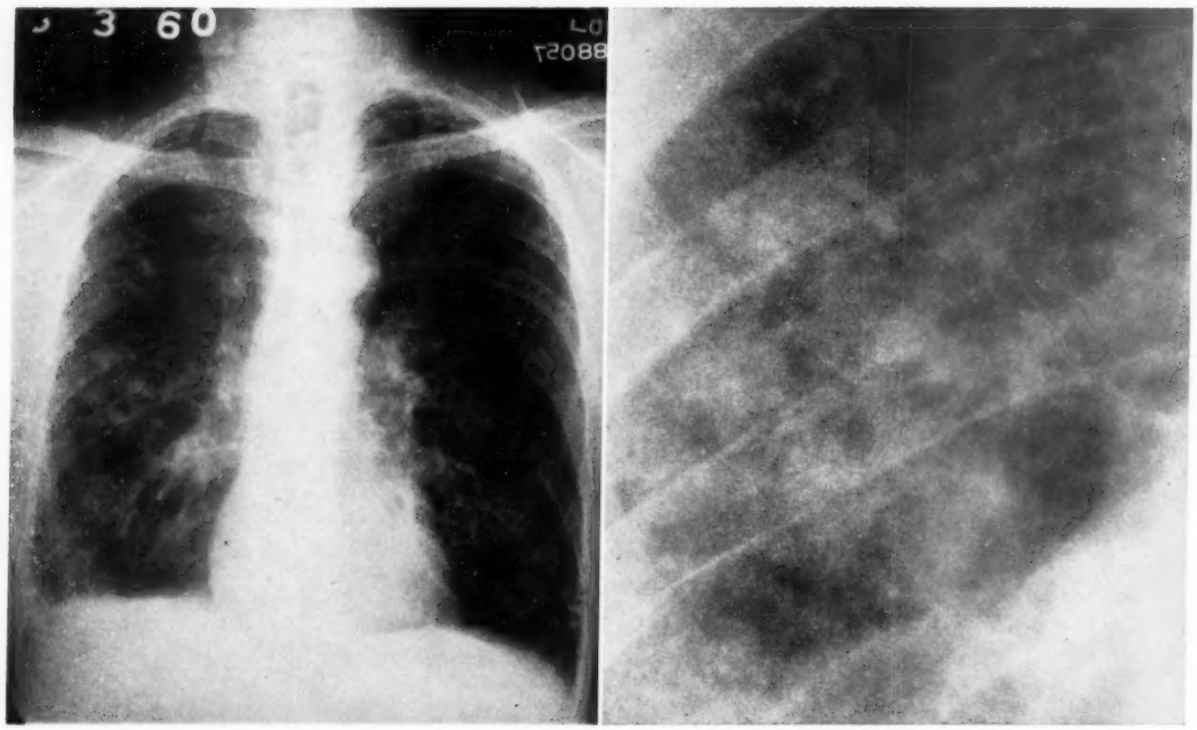


2A 2B

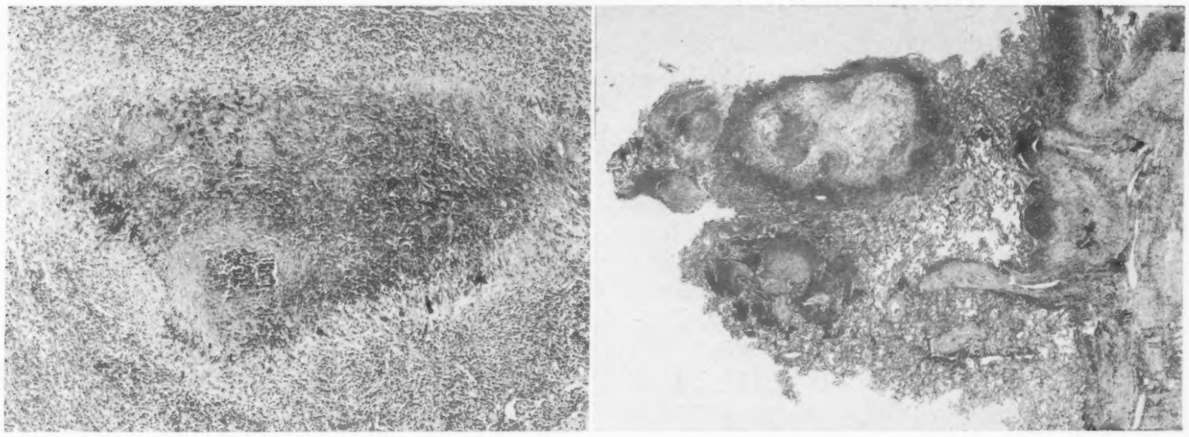
FIG. 2. Roentgenogram (A) and enlargement (B) showing diffuse nodulation involving both lungs.

cluded hemoglobin determinations varying from 8.5 to 10.0 gm. per 100 cc., white blood count 5,500 per cu. mm., hematocrit 29 per cent, sedimentation rate 25 mm. per hour, normal urinalysis, serum uric acid 3.2 mg. per 100 ml., blood urea nitrogen 12 mg. per 100 ml., alkaline phosphatase 3.1 Bodansky units, acid phosphatase 1.2 King-Armstrong units, reactive latex fixation and two-hour postprandial serum sugar 130 mg. per 100 ml. A blood smear showed only a moderate number of microcytes with an essentially normocytic smear. The serum total protein was 6.0 gm. per 100 ml., albumin 1.9, alpha-1 globulin 0.46, alpha-2 globulin 0.86, beta globulin 0.68, gamma globulin 2.08. The first L.E. test showed 1 L.E. cell, no L.E. rosettes, 10 free nuclei, and was considered to be suspicious. The second L.E. test showed 5 L.E. cells, 5 L.E. rosettes, 36 free nuclei, and 4 pre-L.E. cells, and was considered to be definitely positive.

Roentgenograms of his wrists showed bizarre changes consisting of marked soft tissue prominence of both wrists. There was a generalized periarticular demineralization. Actual destruction of the joints was not identified. In the distal ulna there were small cystic changes most prominent on the right and a scooped-out appearance of the articular surface. Definite periosteal new bone was not identified in the shaft area. There was muscle atrophy above the wrists. The chest roentgenogram (Fig. 2) showed the cardiovascular silhouette to be within normal limits. There were rather marked changes in the lungs. These consisted primarily of thickening in the fissures, loss of definition of the right leaf of the diaphragm and chronic scarring in lower lobes of both lungs. There were rather diffuse irregularly sized nodules throughout both lungs, strongly suggesting the possibility of metastatic disease. The root of the right lung was very prominent at its inferior margin and a mass at this point could not be excluded. No definite bony abnormality was noted in the thorax. The radiologist's summary suggested a chronic lung process with superimposed acute pleural changes and pulmonary nodules, most suggestive of a metastatic process and from the appearance of the wrists, some bizarre form of rheumatoid arthritic variant.

A biopsy specimen of the subcutaneous nodules over the left knee was obtained. The sections showed large irregular masses of necrotic, structureless, pink material surrounded by a zone of necrotic nuclear debris which were thin, and beyond these were noted layers of plump fibrocytes lined up perpendicularly to the surface of the necrotic material. There was no evidence of foreign body reaction and no giant cells were noted. The small vessels in the fibrous tissue showed some endothelial hyperplasia and had cuffs of lymphocytes surrounding them. It was concluded that this represented a rheumatoid nodule.

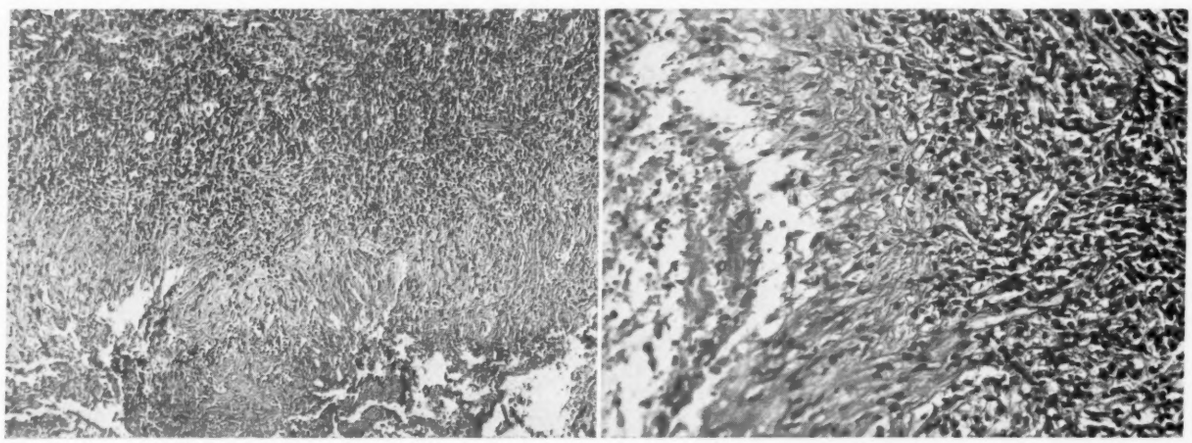
Because of the difficulty in evaluating the pulmonary findings, the patient underwent a diagnostic thoracotomy at which time biopsy material was obtained from the intercostal muscle, pleura and lung.



3 4

FIG. 3. Photomicrograph from the biopsy specimen of the pleura with nodule formation. Hematoxylin and eosin stain, original magnification × 90.

FIG. 4. Low power view of lung showing typical nodular granulomatous lesions with central necrosis, palisading and peripheral cellular infiltration. Hematoxylin and eosin stain, original magnification × 22.



5A 5B

FIG. 5. Photomicrographs of a nodule in the lung also demonstrating an area of central necrosis with surrounding proliferating histiocytic cells showing palisading. Hematoxylin and eosin stain, original magnification × 150 (A), × 390 (B).

The pleura was thickened and a small amount of cloudy pleural fluid was present. Both the middle and lower lobes had easily palpable nodular areas, one of which was excised from the superior segment of the lower lobe. Cultures of its necrotic center were negative for bacteria, including tubercle bacilli and fungi. The biopsy specimen of the intercostal muscle showed no pathologic change.

The biopsy specimen of the pleura, lung and subcutaneous nodule in the chest wall revealed very similar appearing nodular granulomas with central necrosis. (Figs. 3, 4, 5 and 6.) These necrotic areas had fairly sharp borders. The proliferating cells surrounding these central necrotic areas showed some palisading. These proliferating histiocytic cells resembled epithelioid cells. Within the periphery of this layer there was active granulation tissue with newly formed capillaries and active fibroblastic cells. There was in addition considerable inflammatory cell infiltration with many small round cells and plasma cells. Giant cells were absent. The size of these granulomas varied within the different biopsy sites. The lung nodule was a large granuloma, the central zone of which had undergone a little cavitation. Neither fungi nor acid-fast bacilli could be demonstrated on special stains.

While in the hospital the patient was maintained on 50 to 60 gr. of acetylsalicylic acid per day, which gave a salicylate blood level of 22 mg. per cent. Although this gave him satisfactory relief of joint pains he was also given hydroxychloroquine sulfate 200 mg. twice a day.

COMMENTS

The nodule is one of the most important pathologic features of rheumatoid arthritis. Collins [*3*] and Bennett, Zeller and Bauer [*4*] have shown that the characteristic subcutaneous nodule consists of a central area of fibrinoid necrosis, an intermediate zone of fibroblasts in palisade arrangement, and the peripheral zone of cellular infiltration, largely lymphocytes and plasma cells, extending into the surrounding connective tissue.

Similar lesions involving the pleura have been described by Bennett et al. [*4*], Baggenstoss and Rosenberg [*5*], Gruenwald [*6*], Raven, Weber and Price [*7*], Bevans, Nadell, Demartini anb Ragan [*8*], and Ellman, Cudkowicz and Elwood [*9*]. In the latter case the patient also exhibited typical rheumatoid nodulation within the lung adjacent to the pleura.

Rheumatoid lung disease characterized by diffuse non-specific fibrosis has been described by Ellman [*1*], Ellman and Ball [*2*], Yardumian and Kleinerman [*10*], Katz and Auerbach [*11*], Rubin [*12*], Ellman [*13*], Edge and Rickards [*14*], and Brinkman and Chaikof [*15*]; in this last case the patient also demonstrated cystic changes. Price and Skelton [*16*] reported a case in which the blood vessels seemed to be primarily involved.

Caplan [*17*] described a specific type of pneumoconiotic lung nodule in coal miners which is associated in over 50 per cent of the cases with rheumatoid arthritis. True rheumatoid nodules in the lung have been reported only on five previous occasions. Bevans et al. [*8*], reported a case of malignant rheumatoid disease in which typical nodules were found in the lungs at autopsy. Gresham and Kellaway [*18*] described a patient with similar nodules together with cystic changes. Christie [*19*] reported three cases, and Skogrand [*20*] three cases of nodular necrobiotic lung lesions which resembled subcutaneous nodules.

Including the present case and that of Ellman, Cudkowicz and Elwood [*9*], ten cases of non-pneumoconiotic, nodular, necrobiotic pulmonary foci of the rheumatoid type have been reported, all confirmed at autopsy or, as in our case, by lung biopsy. The present case was unusual because so many subcutaneous nodules were present. Also it represents the only case in which a positive L.E. test result has been reported with rheumatoid lung disease. Teilum [*21*] described small granulomatous lesions in lupus erythematosus, but these did not resemble the large necrotizing lesions described here. Since the lung changes seen in lupus erythematosus are non-specific [*22*], more degenerative than inflammatory, and have never been shown to resemble even slightly rheumatoid nodularity, it seems safe to assume that this is a positive L.E. test result associated with rheumatoid arthritis and not with lupus erythematosus itself.

SUMMARY

A patient with nodular rheumatoid lung disease with extensive subcutaneous nodulation and a positive L.E. test is described, in whom the diagnosis was confirmed by lung biopsy. The relevant literature is reviewed.

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Heterotopic Calcification, Hyperphosphatemia and Angioid Streaks of the Retina*

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In view of the heightened interest and active research in fields of connective tissue disorders and genetic transmission of specific enzymatic defects, it seems opportune to report our experience with three patients exhibiting a distinctive combination of associated clinical abnormalities all of which are relatively unusual and may be genetically transmitted, namely, heterotopic calcification, hyperphosphatemia and angioid streaks of the retina.

CASE REPORTS

CASE 1. J. H. (D87240), a forty year old electrician, was seen at Duke Hospital on April 30, 1954, with complaints of impaired vision of thirteen months' duration. At the age of five years a tumor was removed from the left side of his jaw; at nine years of age another tumor was removed from his left hip. Between fourteen and eighteen years of age he underwent seven operations for removal of tumors from various joints (a tumor from the left hip weighed approximately 9 pounds and one from the right hip 4 pounds).

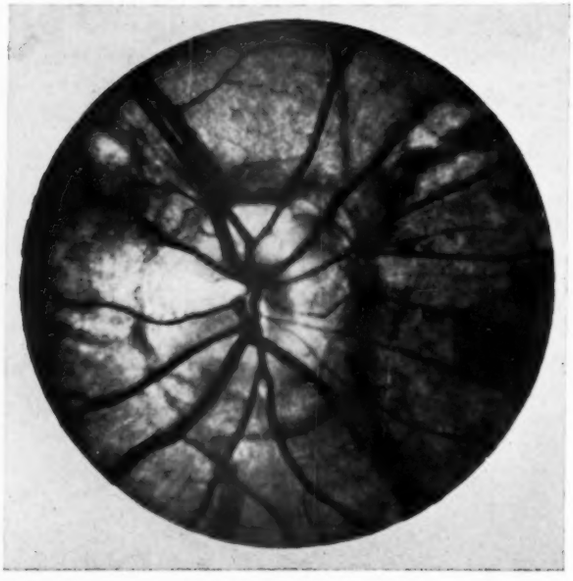

FIG. 1. Case 1. Fundus photo demonstrating the prominent peripapillary and radiating angioid streaks.

His paternal grandfather is said to have had a similar condition involving his chest, fingers and wrists. His father and one sibling had diabetes mellitus. There was no immediate family history of other pertinent illnesses or personal history of injury or illness.

The patient was a wiry white man, with atrophy of the right deltoid muscle in the region of a previous surgical procedure, and numerous scars at operation sites over the right shoulder, elbow, hip, both patellae and lateral aspect of the left foot. There was a 2 by 5 cm. firm mass over the medial aspect of the right elbow and a 1 by 1 cm. mass over the left inferior patellar tendon, as well as a firm 1 by 1 cm. mass in the posterior third of the tongue. The vital signs were normal, blood presssure 140/95 mm. Hg. Examination of the eyes revealed absence of central vision of the left eye, with angioid streaks and macular degeneration bilaterally. (Fig. 1.)

Hemogram and urinalysis were within normal limits repeatedly, as were the serum electrolytes. Other blood chemical determinations are recorded in Chart 1.

X-ray studies revealed no bony abnormalities. The chest and abdominal films were within normal limits. There was diffuse calcification of large and medium-sized blood vessels. Skull films revealed areas of calcification in the petroclinoid ligaments, choroid plexuses and in the region of the basal ganglia of the brain.

The masses over the right elbow and left patellar tendon, the last to appear, were excised. He was treated in the Out-Patient Clinic with probenecid for two months in an attempt to decrease the renal tubular reabsorption of phosphate and to lower serum phosphate levels. There was no change in his clinical status or serum phosphate values. He was

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PATIENT		BENEMID TREATMENT									
		5-1-54	6-27	6-28	6-30	8-10	9-21	11-2	12-4		3-30-59
J.H. Case 1	Calcium	9.2	9.0	9.0			9.5				9.4
	Phosphorus	5.4	3.2	4.6	5.0	5.2	4.4	4.4	5.3		5.2
	Alk P'tase (Bodansky U.)		3.1	2.6							1.3
	Cholesterol	265	315	246			248	233			236
	Uric Acid						2.0				4.8
		1-8-46						1-25-60	2-11-60		
J.V. Case 2	Calcium	9.4						10.4	9.8		
	Phosphorus	7.8						6.3	5.8		
	Alk P'tase (Bodansky U.)	3.1						1.5	1.6		
	Cholesterol								216		
	Uric Acid							4.0	4.0		
								2-11-60	2-27-60		
I.V. Case 3	Calcium							8.8	9.4		
	Phosphorus							5.5	5.0		
	Alk P'tase (Bodansky U.)							1.0	1.1		

CHART 1. Serial determinations of serum calcium and inorganic phosphate in the present cases reported.

re-examined on March 30, 1960. No evidence of soft tissue masses remained but his eyes showed further deterioration. An x-ray survey gave essentially the same findings as in 1954.

CASE 2. J. V. (B67235), a twenty-eight year old Negro farmer, was seen at Duke Hospital for the first time at the age of thirteen years with complaints of an enlarging mass over the medial aspect of his right foot, of several years' duration. The mass occasionally became tender and once was incised, with drainage of chalky material. The mass was excised and the patient discharged. He was re-admitted on February 10, 1960, with complaints of similar large masses appearing over the lateral aspect of his right fifth toe, elbow, shoulder and left hip. These masses would swell periodically, become tender and fluctuant, and drain sanguinous, chalky material spontaneously or upon incision.

The patient was a well developed, slim Negro man with a 3 by 5 cm. mass over his right elbow, a 14 by 9 cm. mass over the left greater trochanter, a 2 by 3 cm. mass over the fourth and fifth metatarsalphalangeal joints, and a 1 by 2 cm. mass over the right acromioclavicular joint. The vital signs were normal, blood pressure 130/80 mm. Hg. Examination of the retinas revealed angioid streaks. (Fig. 2.) There were no skin lesions.

Hemograms and urinalysis were normal, phenolsulfonphthalein excretion 65 per cent in two hours, blood urea nitrogen 5 and 11 mg. per cent, and phosphate clearance 16 ml./minute. Blood mineral determinations are reported in Chart 1; the serum electrolytes were normal. Roentgenograms revealed large amorphous masses of calcium over the left hip (Fig. 3), right shoulder, elbow and fifth metatarsalphalangeal joint. Skin biopsy specimens from axilla and neck were normal with routine and elastic stains.

The masses, involving all tissue planes in the areas mentioned, were excised under general anesthesia

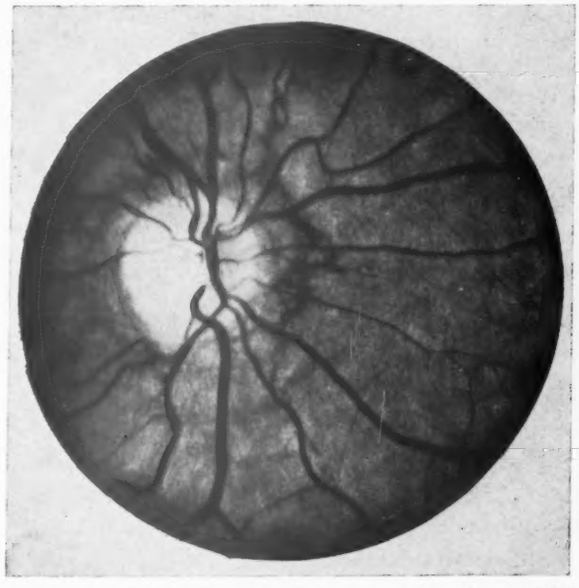


FIG. 2. Case 2. Fundus photo demonstrating the angioid streaks radiating from the peripapillary area.

FIG. 3. Case 2. Roentgenogram of left hip illustrating the heavy deposition of calcium salts in the soft tissues of that area.

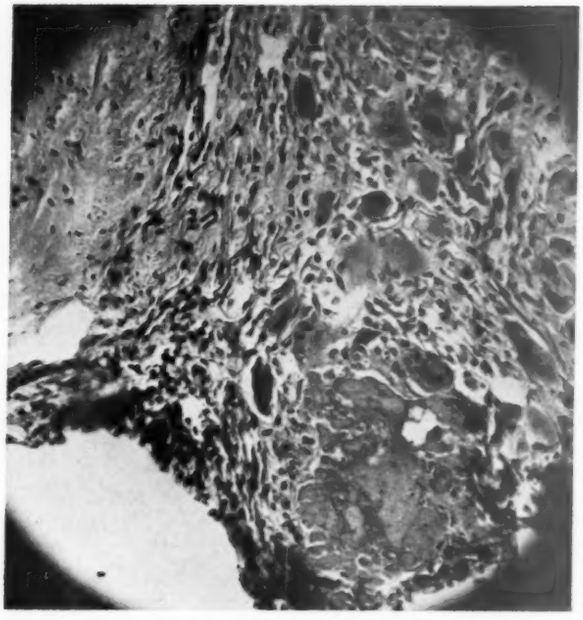


FIG. 4. Case 2. High power photomicrograph of surgical specimen resected from area of left hip, demonstrating the calcific deposit, fibroblastic response and numerous giant cells.

and, except for postoperative drainage from two sites, the patient's course was uncomplicated. He was discharged and directed to follow a low phosphate diet.

CASE 3. I. V., brother of J. V. (Case 2), a thirty-two year old farmer, was examined on March 26, 1960. He had a history of numerous surgical excisions of large calcific masses over hips, elbows and shoulders approximately twenty years before on different occasions, finally without recurrence. The review of systems was negative. As did the other patients, he denied ingestion of large amounts of milk or alkali, vitamin D, or symptoms of hypercalcemia.

He was a small, well developed Negro man with normal vital signs, blood pressure 110/70 mm. Hg. Examination revealed marked angioid streaks of the retina and numerous scars at the sites of surgical excisions mentioned.

Hemogram and urinalysis were within normal limits. Blood chemical studies are recorded in Chart 1. Skin biopsy specimens of axilla and neck were normal with routine and elastic stains. X-ray survey revealed large amorphous deposits of calcium around the right hip.

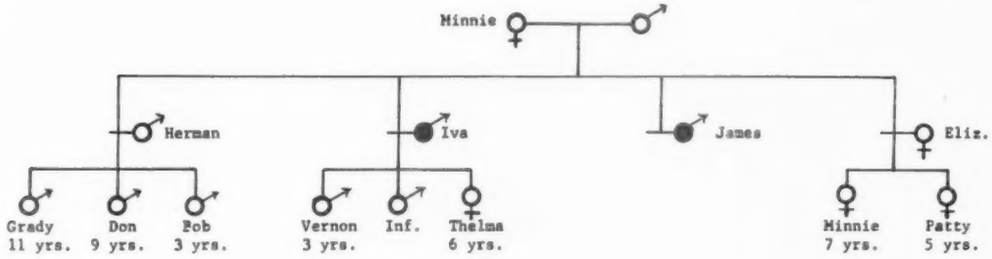
PATHOLOGIC EXAMINATION

The surgical specimen in Case 2 revealed it to consist principally of amorphous calcium deposits with some areas of dense fibrosis and granulomas of epithelioid and foreign body giant cells surrounded by a lymphocytic infiltration. (Fig. 4.) Review of the sections from the excised masses in Case 1 disclosed a similar microscopic pattern of giant cells, fibrosis and heavy deposits of calcium. The fibrous tissue was dense and rather acellular. In some areas there were small groups of macrophages with foamy cytoplasm.

Review of surgical biopsy specimens in four cases of so-called calcinosis universalis seen at Duke Hospital revealed essentially the same histopathologic pattern, without significant evidence of fatty degeneration or fat necrosis. Others [*11,16*] have noted this histopathologic pattern of calcium deposition, giant cell reaction and intense fibrosis in cases of calcinosis. Loria et al. [*3*] observed that calcium salts tend to precipitate in areas of elastic fiber degeneration in pseudoxanthoma elasticum, as shown by electron microscopy. Chemical analysis of the calcific deposit in Case 2 indicated that only calcium phosphate was present in significant amounts.

FAMILY BACKGROUND

In view of the familial occurrence of pseudoxanthoma elasticum and Paget's disease [*4,5*], systemic diseases [*6*] which are associated with angioid streaks of the retina and calcinosis, a limited study of the family of patients J. V. and I. V. (Cases 2 and 3) was carried out. Medical history, physical examination, blood mineral and hematological studies were performed in



	James	Iva	Herman	Eliz.	Minnie	Grady	Don	Bobby	Vernon	Inf.	Thelma	Minnie	Patty
Calcium	9.7,10.4,9.8	8.8,9.4	7.9	9.3	9.4	10.2	9.6		10.0		9.9	9.9	9.4
Phosphorus	7.3,6.3,5.8	5.5,5.0	3.3	3.1	3.0	4.1	3.4		5.7		4.9	4.9	4.7
Alk P'tase	3.7,1.5,1.5	1.0,1.1	0.9	1.7	2.4	4.1	4.4		3.4		3.6	4.7	5.5
Cholesterol	216			162	252								
Uric Acid	4.0	4.8	3.8	4.8	4.0	2.5							
Hemoglobin Electrophor.	14.0 gm.% AA	AA	AA	AA		12.0gm	11.2		11.2		13.9	11.0	12.0
Sickle Prep.	neg.				neg.	neg.	neg.		neg.		neg.	neg.	neg.

CHART 2. Family tree of Cases 2 and 3, with blood chemical data. ♂ represents affected siblings.

each case, as well as appropriate x-rays and skin biopsies. No other members were found to have angioid streaks of the retina, skin lesions or calcinosis. (Chart 2.)

The immediate family of patient J. H. (Case 1) was not available to study because of geographical scatter, however his paternal grandfather is reputed to have had a similar condition and this family has very distant kinship with the H. family reported by Anderson [*7*] as a family of pseudoxanthoma elasticum with angioid streaks and choroidal sclerosis of the eyes. There is no known case of heterotopic calcification in the latter family save for one member whose skull roentgenograms showed heavy calcification in the falx and choroid plexuses.

COMMENTS

A variety of terms has been used to describe abnormal deposition of calcium salts in soft tissues: pathological calcification, calcinosis, heterotopic calcification, and others. These descriptive terms are frequently used in an attempt to classify the calcification in terms of underlying etiology, metabolic disturbance or according to the location of the deposits. They are frequently misleading and often the distinctions are altogether artificial. As Wheeler [*1*] points out, careful investigation of such cases usually incriminates a distinct underlying disease entity, such as collagen diseases, hypercalcemic states, chronic renal insufficiency, and the like.

The heterotopic calcification in our patients is but one feature of their illness, and not necessarily the most important, but by far the most striking. Massive calcification as such is not a specific entity and is relatively uncommon, but is known to occur in chronic renal disease [*8*], sarcoidosis [*9*], in association with secondary amyloid deposition [*10*], following trauma [*11*], and in otherwise apparently normal people [*11*].

Certain cases of massive heterotopic or tumoral calcification have been reported in siblings. Cole [*2*] points out that in this group of tumoral calcification the massive deposition of lime salts sometimes is self-limited, occurs in young adults and carries a good prognosis. In none of the reported cases have angioid streaks or a family history of pseudoxanthoma elasticum been noted.

Hyperphosphatemia has been an inconstant feature of other reported cases of heterotopic calcification [*12–16*]. However, in many of the earlier studies chronic renal disease was present; in others, whole blood phosphate was measured and phosphate determinations were not performed by comparable technics. However, if we limit our remarks to cases of heterotopic

calcification with normocalcemia and without renal insufficiency there still remain a few cases of unexplained hyperphosphatemia [*12,13,19*]. Metabolic balance studies have been carried out in patients with calcinosis or heterotopic calcification but the results are conflicting [*18*]. Bauer et al. [*17*] reported a positive balance for calcium and phosphorus on a very low intake, as did Brooks [*14*], but Atkinson [*15*] was of the opinion that so small a positive balance in such cases was not enough evidence to justify the concept that abnormal mineral metabolism is an etiologic factor in these cases.

In our own cases, as in others cited, there is no evidence of other causes of hyperphosphatemia, such as hypoparathyroidism, pseudohypoparathyroidism, renal failure, dietary idiosyncrasy, hypophosphatasia, or increased growth hormone activity, nor was there any demonstrable abnormality of calcium metabolism. Due to circumstances beyond our control we were unable to perform studies at the time to attempt to elucidate the mechanism of hyperphosphatemia. A review of the records of thirteen patients with pseudoxanthoma elasticum observed at Duke Hospital reveals that seven have had measurements of calcium and phosphorus, all of which were normal.

Perhaps as more cases are collected it may become clear that hyperphosphatemia is an important facet in this association of signs and symptoms, and that it is genetically determined. It is also possible that our three cases represent heterotopic calcification in reaction to some connective tissue disorder which is a latent, or forme fruste, of pseudoxanthoma elasticum. Angioid streaks have been reported in association with sickle cell disease. There was no evidence of hemoglobinopathy in the three cases herein reported.

SUMMARY

Three cases of massive heterotopic calcification associated with angioid streaks of the retina and hyperphosphatemia are reported and discussed. So far as we are aware this is the first reported association of these abnormalities.

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Recurrent Ventricular Tachycardia*

A Case Successfully Treated by Bilateral Cardiac Sympathectomy

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The following case is of interest not only because of the successful employment of bilateral cardiac sympathectomy in ventricular tachycardia, the first such case to be reported, but also because of the unusual nature of the tachycardia, its refractiveness to usual therapeutic measures, and because of the effect of the surgical procedure on intraventricular conduction.

CASE REPORT

This thirty-five year old grocery clerk was first admitted to the Durham Veterans Administration Hospital on June 23, 1953, having been transferred from another hospital with a diagnosis of recurrent paroxysmal ventricular tachycardia.

The patient was well until October 1952, at which time he had a tight substernal discomfort associated with weakness and dyspnea. He was hospitalized at another hospital where a diagnosis of ventricular tachycardia was made. This was controlled with medications but soon recurred. Recurrences appeared without relation to activity or other events and lasted from a few seconds to seventy-two hours. The frequency and duration of these attacks necessitated his almost continuous hospitalization during the nine-month interval between the onset of symptoms and his first admission here. Medications during this period included quinidine and procaine amide given in massive doses, ACTH, rauwolfia derivatives, heavy sedation and digitalis. Between attacks of ventricular tachycardia, a heart murmur was heard which led to a diagnosis of rheumatic heart disease with mitral insufficiency. Because of the persistence of the attacks and his gradual downhill course, he was transferred to this hospital.

The patient recalled no unusual childhood illnesses. He led a healthy and active childhood. He was accepted into the army in January 1944 and sent to a training camp, where he did amateur boxing. On one occasion, prior to a bout, the examining physician heard a heart murmur and cancelled the bout. The patient was referred to the hospital for further study, although he had no symptoms. He was found to have a blowing systolic apical murmur with transmission to the axilla, and a short presystolic apical murmur was also described. Chest films showed a prominent pulmonary artery. The electrocardiogram was normal. He was discharged from the army in May 1944, the discharge diagnosis being valvular heart disease with mitral insufficiency, and mitral stenosis, presumably rheumatic. The patient returned to fulltime work and remained symptom free until the onset of the present illness.

The patient's mother died suddenly of an unknown cause in her sixties; his father died in his seventies of "heart dropsy."

The patient weighed 180 pounds on admission (weight prior to illness 210 to 220 pounds). He was afebrile; the pulse rate was 82 per minute; the blood pressure 125/85 mm. Hg. He was alert, cooperative and comfortable on admission. The ocular fundi were normal. There was no lymphadenopathy. The lungs were normal. There was a basic regular heart rate of 82 beats per minute, but this was often interrupted by premature beats and runs of regular tachycardia at a rate of 144 per minute lasting from several beats to thirty seconds. The heart was normal in size and a grade 1 basal systolic murmur was heard. No diastolic murmurs were made out. The peripheral vessels were normal. The remainder of the physical examination was non-contributory.

During the attacks of tachycardia the patient appeared to be in some distress and complained of a dull substernal oppression. Vagal stimulation had no effect on the tachycardia. Laboratory data were all within normal limits except for a mild elevation of white blood count to 13,900 per cu. mm. with 75 per cent polymorphonuclear leukocytes, 4 stab forms, 19 lymphocytes, 2 eosinophils. Chest film and fluoroscopy on admission revealed no cardiomegaly and no specific chamber enlargement. Initial electrocardiogram showed a right bundle branch block, a first degree A-V block, multiple premature ventricular beats and runs of ventricular tachycardia.

On admission all medications were stopped, without apparent effect on the frequency or character of the

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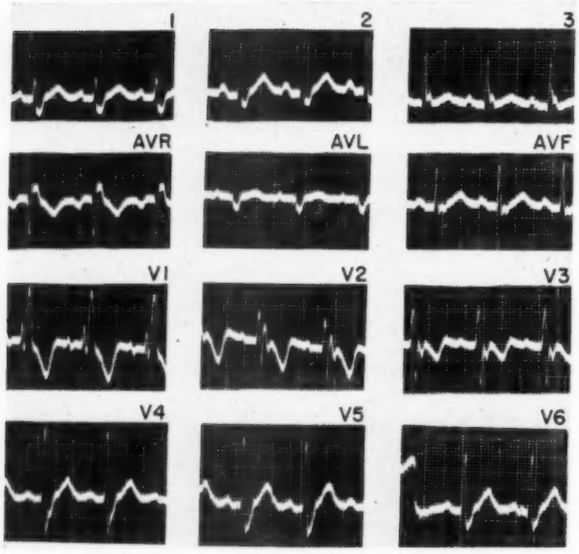

FIG. 1. An electrocardiogram taken four months prior to admission (February 27, 1953). The electrocardiogram taken on admission was identical except for the presence of multiple premature beats.

episodes of tachycardia. Since the patient had had adequate trials on quinidine and procaine amide in previous hospitals, the following drugs were tested over the next few weeks: (1) potassium chloride, 8 gm. per day given orally, (2) intravenously administered magnesium sulfate, (3) atabrine given intramuscularly in doses up to 1 gm. per day, (4) propylthiouracil, 600 mg. per day given orally.

The attacks became gradually more prolonged—one run lasting for 112 hours. More substernal distress began to develop during the attacks, and during attacks blood pressure dropped to 85/60 mm. Hg, the liver became palpable and basal rales appeared. Digitalization failed to relieve these signs or to influence the course of the tachycardia. The administration of mercurial diuretics and a sodium restricted diet became necessary because of fluid retention during the attacks of tachycardia.

On September 27, 1953, a left dorsal sympathectomy was performed, with removal of the stellate ganglion and the upper six thoracic ganglia. The patient had a run of tachycardia during the procedure but the following day he maintained a normal sinus rhythm. On the second postoperative day he had a six-hour run of tachycardia. Over the next three days he had recurrent episodes which became shorter in duration. After this the tachycardia ceased; he was allowed gradually to increase his activities, and was discharged on October 30, 1953, on a regimen of quinidine, 0.4 gm. orally four times daily. He experienced only occasional premature ventricular beats during his last few weeks of hospitalization.

He returned in December 1953 at which time he was found to have occasional auricular premature contractions. The electrocardiogram showed a reduction in QRS conduction time from 0.14 to 0.10 second. Incomplete right bundle branch block was present. The P-R interval was normal.

In October 1954 the patient was well and had stopped taking quinidine. An electrocardiogram showed a normal QRS conduction time of 0.08 second, the electrical axis was horizontal, the R′ had disappeared in lead V_1.

In January 1955 he experienced a ten-hour run of tachycardia. Thereafter, he had one or two short runs per week, but these did not limit him in any way. In September 1955 he had pleuritic pain in the right side of his chest for one week. Laboratory and x-ray studies failed to establish a cause for these symptoms. In January 1956 a prolonged period of tachycardia developed with the same symptoms experienced originally and the patient was hospitalized briefly in another hospital. An electrocardiogram revealed recurrence of the right bundle branch block with a QRS duration of 0.12 second. He was readmitted to the Durhams Veterans Administration Hospital on January 27, 1956, for re-evaluation. On February 7, 1956, a prolonged episode of tachycardia developed and ventricular tachycardia was documented electrocardiographically. On February 16, 1956, a transthoracic right dorsal sympathectomy was performed. The postoperative course was uneventful and he was discharged on March 1, 1956.

The patient was last seen in April 1960. At this time he reported occasional short runs of tachycardia, usually about once a month, lasting for five to ten minutes, but not limiting him in any way. His only complaint was that of occasional wheezing respirations, worse with changes in weather, and mild exertional dyspnea. He was working as a ticket seller. Examination revealed him to be in good spirits, appearing well. There was a soft, systolic basal murmur, otherwise the physical examination was completely within normal limits. The heart size was normal on the chest roentgenogram. The electrocardiogram again showed a normal QRS conduction time of 0.08 second.

Electrocardiographic Findings. Electrocardiograms were forwarded by the patient's referring physician, and showed a right bundle branch block between attacks of tachycardia. (Fig. 1.) The QRS conduction time ranged between 0.12 and 0.14 second. The P-R interval was 0.20 second. The attacks of tachycardia were characterized by two distinct forms of QRS conduction. One form resembled a right bundle branch block with a ventricular rate of about 160 beats per minute; the other a left bundle branch block with approximately the same ventricular rate. P waves could not be definitely identified on the electrocardiograms forwarded with the patient.

Soon after admission to this hospital electrocardiograms were taken which again revealed a right bundle branch block between episodes of tachycardia. On

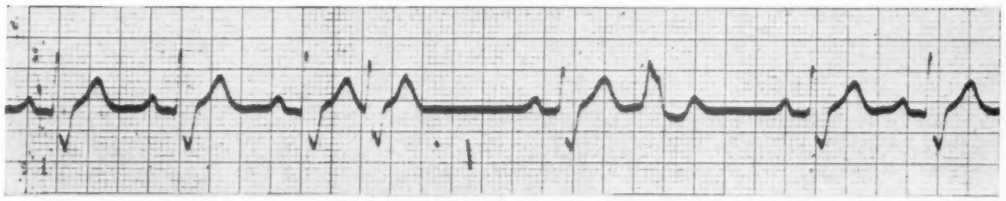

FIG. 2. Lead I, taken on admission (June 23, 1953), showing premature beats resembling each of the two types of tachycardia in the same lead. Neither premature beat interrupts the basic auricular rhythm.

admission there were frequent premature beats which varied considerably in character. These premature beats did not interrupt the auricular rhythm, and were followed by a complete compensatory pause. The P wave could often be identified in the T wave of the premature beat. On some leads premature beats were seen which resembled each of the two conduction patterns seen with the attacks of tachycardia. (Fig. 2.)

Soon after admission electrocardiograms were recorded with both of the types of paroxysmal tachycardia. Esophageal leads revealed that both types were accompanied by complete A-V dissociation. (Fig. 3.) The QRS duration with both types of tachycardia was 0.12 to 0.14 second. (Figs. 4 and 5.)

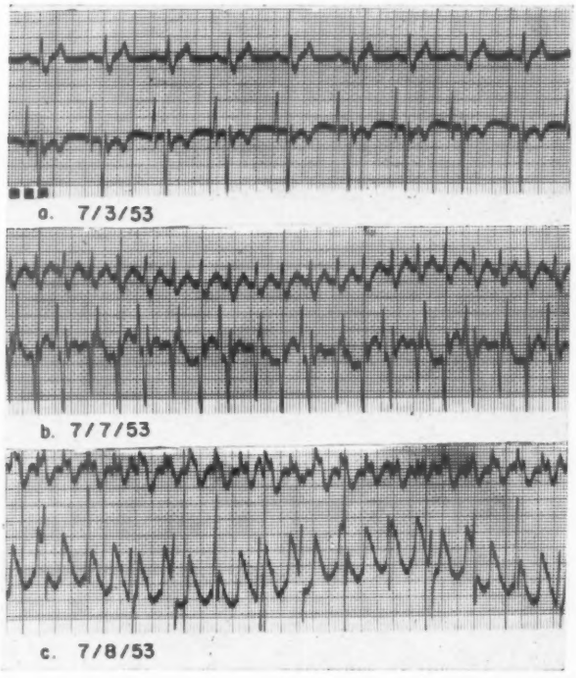

FIG. 3. Lead I (above) and simultaneous esophageal lea (below) taken during (A) normal conduction (July 3 1953), (B) the "right bundle" type tachycardia (July 7. 1953) and (C) the "left bundle" type tachycardia (July 8, 1953). Both (B) and (C) show independent atrial and ventricular activity. The P waves are the sharp upward spike in the esophageal lead in all tracings.

Subsequent preoperative electrocardiograms always revealed the findings described. Between attacks of tachycardia there were frequent premature beats which usually had the characteristics of "main stem" extrasystoles [*1*], having the QRS configuration of the sinus beats, but not interrupting the auricular rhythm, and followed by a complete compensatory pause.

After the first operative procedure in September 1953 there was a change in QRS conduction, the conduction time being shortened to 0.10 second. (Fig. 6.) There were still occasional premature ventricular contractions, which were now clearly more prolonged than the sinus beats, reaching 0.14 second in duration. The sinus beats still showed abnormalities in conduction; an S wave in leads I, II and III and an R′ in lead V_1. An electrocardiogram in January 1954 revealed a QRS conduction time of 0.10 second. S waves were present in leads I, II and III, but these were much less striking in size than on previous records. The R′ in lead V_1 had entirely disappeared. During 1955, coincident with the return of attacks of tachycardia, a very small R′ again appeared in lead V_1 and QRS conduction became prolonged to 0.12 second.

In January 1956 the electrocardiogram showed a more prominent S wave in leads I and II and a more prominent R′ in lead V_1. Again "main stem" extrasystoles were seen. After the second operative procedure the electrocardiogram once more showed a reduction in QRS conduction time to 0.10 second

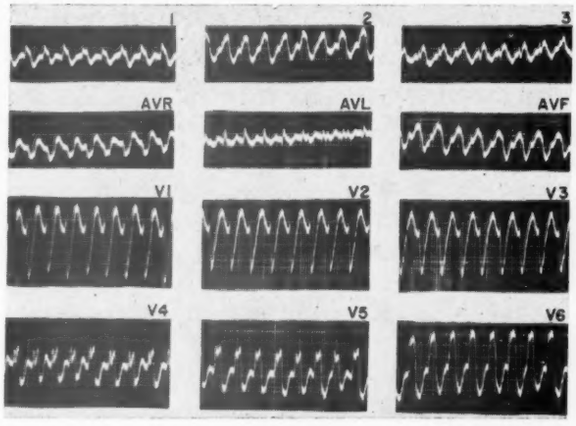

FIG. 4. Electrocardiogram showing the tachycardia resembling left bundle branch block (February 27, 1953).

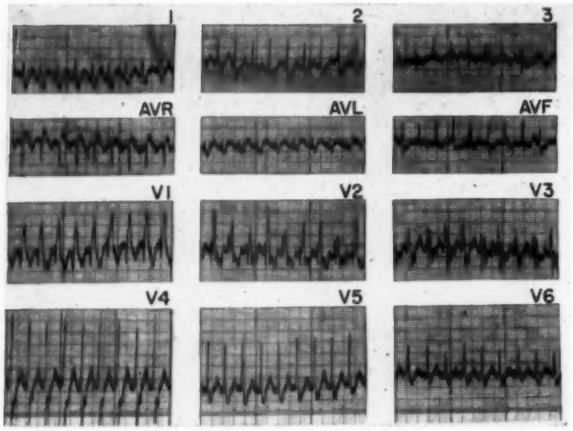

FIG. 5. Electrocardiogram showing the tachycardia resembling right bundle branch block (July 7, 1953).

and again the R′ began to diminish in size. It was still present on March 14, 1956. On September 22, 1958, the electrocardiogram revealed a normal QRS conduction time, a P-R interval of 0.16 second, a QRS duration of 0.08 second with no evidence of R′ in lead V_1.

COMMENTS

The difficulty in establishing a diagnosis of ventricular tachycardia is recognized. This is especially true when the tachycardia has a configuration so similar to the sinus beat, as in the runs of tachycardia in which QRS complexes resembled right bundle branch block. No fusion beats were seen and at no time could capture beats be identified, therefore the tachycardia cannot be identified with certainty as ventricular tachycardia. However, such does seem to be the case since (1) there was complete A-V dissociation with both forms of ventricular tachycardia; (2) premature beats could be seen which resembled both types of tachycardia; (3) the premature beats resembling the sinus beats were followed by a P wave which timed perfectly with the basic auricular rhythm. These premature beats were followed by a complete compensatory pause and can be considered "main stem" extrasystoles of high ventricular origin or low nodal extrasystoles with retrograde block.

Although the ventricular origin of the tachycardia resembling left bundle branch block also cannot be proved with certainty, there is less doubt of its character because of the bizarre form of the QRS complexes, totally different from the sinus beats.

Thus there is evidence in favor of ventricular tachycardia as a mechanism for both forms of

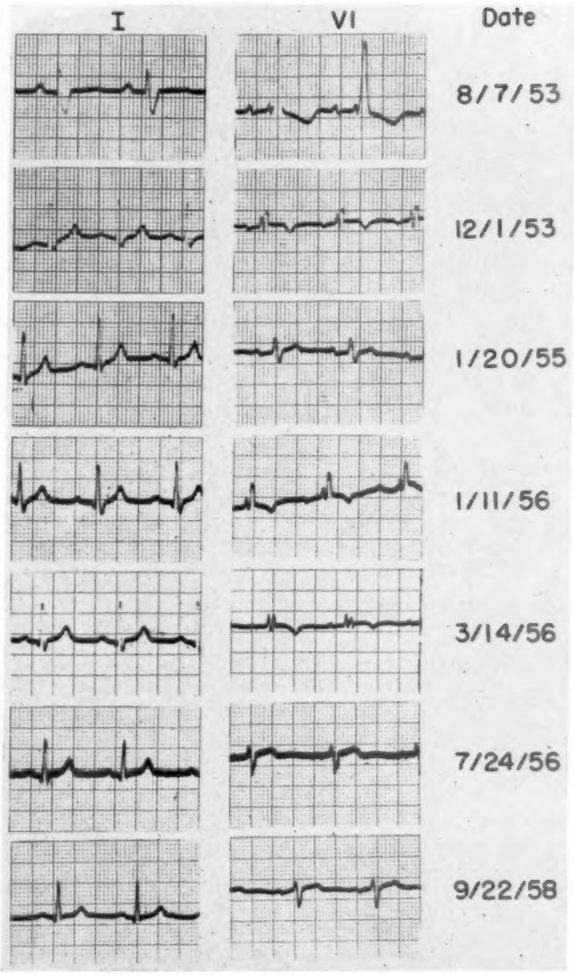

FIG. 6. Representative strips of leads I and V_1 showing the change in conduction during the course of observation. The left thoracic sympathectomy was performed between the first and second strips, the right sympathectomy between the fourth and fifth strips.

tachycardia, although this cannot be proved beyond reasonable doubt.

Bilateral cardiac sympathectomy has been used a number of times for treatment of tachycardia of supraventricular origin. The first such case was reported by Leriche and Fontaine in 1929 in a case of auricular tachycardia [*2*]. Almost all the subsequent reports have appeared in the French literature. The last and most comprehensive paper, describing six patients so treated, was that of White and Bland [*3*].

A number of studies in animals have shown that induced ventricular extrasystoles can be stopped by removal or blockade of the sympathetic nerve supply [*4–6*]. It has also been noted that adrenalin and sympathetic stimulation promote ectopic escape mechanisms [*7,8*].

Goodall and Kirshner [*9*] have shown that, in dogs and sheep, interruption of the sympathetic fibers to the heart by means of bilateral thoracic sympathectomy reduces the myocardial norepinephrine content to about one-sixth of its former level. This reduction was of transient nature, however, the level beginning to rise at about five weeks and returning to normal at sixteen weeks.

It appears that epinephrine, norepinephrine and sympathetic stimulation are conductive to the appearance of ventricular escape beats or ventricular extrasystoles. Interruption of the sympathetic fibers reduces the tendency for ectopic beats in the experimental animal. Interruption of the sympathetic nervous system reduces the content of norepinephrine in cardiac muscle. The possible significance of such reduction in the improvement experienced by the patient is uncertain, since there is a return to control level in five to sixteen weeks.

The present case illustrates the fact that bilateral cardiac sympathectomy can be a valuable therapeutic measure in treatment of paroxysmal ventricular tachycardia. It is of course recommended that more conventional methods of therapy be tried first, but if these fail cardiac sympathectomy would seem to be a reasonable next step.

Also of interest is the change in QRS conduction after each of the operative procedures in this case. This suggests that there might have been some influence of the sympathetic nervous system on the choice of conduction pathway through the A-V nodal and junctional tissue.

SUMMARY

A patient with a refractory case of recurrent ventricular tachycardia is described, with striking improvement after bilateral cardiac sympathectomy. The operative procedure was followed by normalization of a prolonged QRS conduction time.

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Hyperparathyroidism Due to Late Recurrence of Parathyroid Adenoma*

Report of Two Cases

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IN 1903 Askanazy [*1*] discovered a parathyroid tumor in a patient dying of von Recklinghausen's disease of bone and thereby equated, for the first time, a parathyroid adenoma with hyperparathyroidism. Later, in 1934, primary clear (wasserhelle) cell hyperplasia involving all four glands was described as a cause of hyperparathyroidism by Albright et al. [*2*]. More recently, Cope et al. [*3*] have described what they term "primary chief cell hyperplasia," in which the cells are chief cells and all four glands are involved, a condition readily confused with an adenoma when only one gland is examined. This disorder may be associated with other endocrine abnormalities such as an islet cell adenoma of the pancreas or pituitary tumors. Parathyroid carcinomas and secondary parathyroid hyperplasia are also known to cause hyperparathyroidism. Multiple parathyroid adenomas as a cause of hyperparathyroidism are well documented in the literature [*4–6*]. They are usually removed together at the first operation or at a subsequent operation because symptoms fail to improve after the initial removal [*7,8*].

On the other hand recurrent hyperparathyroidism due to recurrence of a solitary parathyroid adenoma is rare, judging from the scant reports in the literature. Recently we have seen two cases of recurrent adenomas occurring six and eighteen years, respectively, after the first episode.

CASE REPORTS

CASE 1. Mrs. E. A., a Jewish housewife, first came to the attention of the Royal Victoria Hospital in 1952 at the age of twenty-nine. Two years previously she had had an attack of mild renal colic due to "gravel," and a more severe renal colic three months prior to admission. She was admitted to the hospital for investigation of possible hyperparathyroidism. At that time she stated that for five years she had noted swelling of both hands and ankles as well as the lower parts of both legs, occurring in episodes lasting for about one to one and a half days. The swelling was most pronounced just prior to the menses, during the summer months, and during the two attacks of renal colic. Her menstrual periods over the previous year had decreased to one day from her usual flow of three to four days. She also noted easy irritability, tiredness, excessive perspiration of her hands and feet, slight exertional dyspnea and, for the previous three years, peculiar twitching sensations of her abdominal wall.

Physical examination revealed a somewhat obese white woman in no apparent distress. The pulse was 96 per minute and regular, the blood pressure was 130/90 mm. Hg. The skin was warm and moist. Her hands and feet appeared somewhat edematous, but this was not pitting in type. Otherwise the physical examination was within normal limits.

Laboratory investigations revealed a normal urinalysis with an acid pH, specific gravity of 1.017 and negative reaction to tests for sugar and albumin. The hemoglobin was 14.7 gm. per cent, the erythrocyte sedimentation rate was 9 mm. per hour and the white cell count was 10,300 per cu. mm. The serum non-protein nitrogen was 34 mg. per cent, the serum total proteins 6.7 gm. per cent with an albumin of 5.1 gm. per cent and a globulin of 1.6 gm. per cent. Results of liver flocculation tests were normal. Serum calcium values were 12.8, 13.4 and 13.5 mg. per cent, with serum inorganic phosphorus values of 2, 2 and 2.3 mg. per cent, respectively. The serum alkaline phosphatase was 7.3 and again 6.3 King-Armstrong units (normal, 3 to 13 King-Armstrong units). X-ray examination of the abdomen revealed the presence of a single radiopaque shadow projected over the upper pole of both kidneys, measuring at least 6 mm. in diameter. Otherwise the general skeletal survey

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revealed no bony abnormalities, and no abnormalities of the lamina dura were noted.

On April 23, 1952, the patient was subjected to an exploratory operation which revealed a large parathyroid tumor, measuring 12 mm. in diameter, lying on the posterior surface of the mid-point of the right lobe of the thyroid between the carotid sheath and the posterior surface of the esophagus. No other parathyroid tumors were found. The histologic diagnosis was a parathyroid adenoma which showed several cystic areas filled with blood and macrophages. The glandular cells were arranged in nests and anastomosing cords, with thin-walled capillaries intervening. The cells varied in size and appearance; the cells at the periphery were smaller, with round dark central nuclei and a moderate amount of pale vacuolated cytoplasm; those at the centre were larger with slightly bigger nuclei and abundant foamy pale acidophilic cytoplasm. No mitoses were seen and pleomorphism was not marked. (Fig. 1.)

The postoperative course was uneventful and the patient did not require any calcium or vitamin D therapy. Postoperative serum calcium levels during the following week were 9.2, 8.9, 9.1 and 10.3 mg. per cent, with serum inorganic phosphorus levels of 3, 3.1, 4 and 3.8 mg. per cent and alkaline phosphatase of 8.1, 6.6, 7.8 and 7 units. Following discharge, a right ureterolithotomy was performed at another hospital. She remained well, and in September 1953 gave birth to a healthy baby girl, without complications. The patient continued in good health until September 1957 when she was taken to another hospital with acute left ureteral colic; left ureterolithotomy was performed. The serum calcium at this time was 10.4 mg. per cent and the serum inorganic phosphorus 2.4 mg. per cent. Further investigation during the next year performed at this same hospital revealed serum calcium levels of 11.2 and 13 mg. per cent, serum phosphorus levels of 2, 2.4 and 2.8 mg. per cent and a single alkaline phosphatase determination of 5 units. Tubular reabsorption of phosphorus was 89 per cent. Urinary calcium excretion while the patient was on an uncontrolled diet showed values of 375, 225 and 525 mg. per twenty-four-hour volume of urine.

Because of these findings the patient was readmitted to the Royal Victoria Hospital in September 1958 for further study. At this time the patient revealed that she had not felt well since the episode of renal colic in 1957. She had also noted the onset of edema of the ankles which was quite severe in nature, but which improved after a few weeks. Edema of the ankles recurred again in June 1958, but one month prior to this final admission had subsided again. Associated with the ankle edema she had noted some generalized puffiness. There had been a weight gain of 15 to 20 pounds during the preceding year. Since the attack of renal colic there had been a decrease in her menstrual flow from the usual four to five days to one day per cycle. She denied any polyuria or polydipsia, but had noted increased perspiration and occasionally awoke to find her night clothes drenched with sweat. Since June 1958 she experienced extreme tiredness to the point of complete exhaustion. The fatigue was present throughout the day, but reached its maximum in the late afternoon. She also noted a marked personality change during this period. She had become irritable, nervous and moody, and was unable to concentrate on her work. She had also experienced some exertional dyspnea after climbing one flight of stairs or on walking long distances.

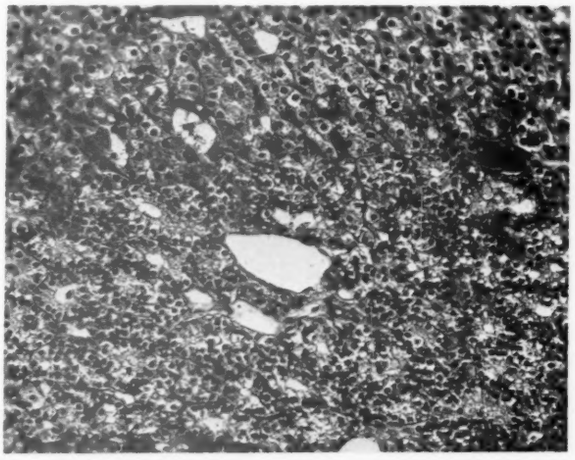

FIG. 1. Case 1. Histologic section of the first parathyroid adenoma removed in 1952.

Physical examination revealed a fairly obese thirty-six year old white woman in no apparent distress. The pulse was 88 and regular, the blood pressure 130/80 mm. Hg. The neck showed a well healed transverse scar of the previous parathyroidectomy. No masses were palpable in the neck. There were well healed ureterolithotomy scars bilaterally. The arms felt puffy without any pitting and there was a trace of pitting edema of both legs. Otherwise the physical examination was non-contributory.

Laboratory investigations revealed a urinalysis with a specific gravity of 1.025, pH 5.5, no sugar or albumin, 10 to 12 white blood cells and occasional red cells on microscopic examination, and a 2- to 3-plus reaction to the Sulkowitch test. The hemoglobin was 14.9 gm. per cent, the hematocrit 45 per cent, the sedimentation rate 6 mm. per hour with a white blood cell count of 9,600 per cu. mm. The serum nonprotein nitrogen was 18 mg. per cent. Serum calcium levels were 11.2, 11.5, 11.3 mg. per cent and the serum inorganic phosphorus levels were 2.2, 2.2, 2.3 mg. per cent with alkaline phosphatase values of 3, 3.6 and 3.6 King-Armstrong units. Urinary calcium excretion while the patient was on a low calcium diet (115 mg. of calcium per day) was 334, 180, 312, 288, 206 and 230 mg. per twenty-four-hour urine volume. Tubular reabsorption of phosphorus on two occasions measured 87 and 95 per cent.

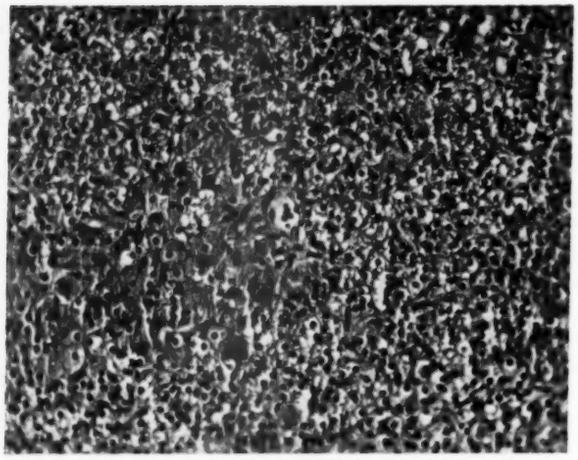

FIG. 2. Case I. Histologic section of the second parathyroid adenoma removed in 1958.

An intravenous pyelogram demonstrated a calculus in the body of the left kidney and a small calculus situated in the right ureter about 4 to 5 cm. distal to the ureteropelvic junction and not causing obstruction to the egress of urine. A general skeletal survey showed no evidence of bone disease. No generalized areas of rarefaction or any cystic changes were evident. The lamina dura was intact.

On September 20, 1958, the patient underwent an exploratory operation. A well encapsulated parathyroid tumor measuring 5 by 8 mm. was discovered and removed from the posterior aspect of the left thyroid gland, just above the inferior pole. On microscopic examination the tissue consisted almost entirely of cells showing pale and occasionally foamy cytoplasm and dark-staining pyknotic nuclei. No mitotic figures or atypical cell forms were seen. The histologic diagnosis was that of parathyroid adenoma. (Fig. 2.)

The postoperative course was uneventful except for a transient Chvostek's sign on the third postoperative day. This disappeared rapidly and no therapy in the form of calcium or vitamin D was required. Daily postoperative serum calcium levels were 9 to 9.7 mg. per cent and serum phosphorus levels were 3.6 to 4.5 mg. per cent. The serum alkaline phosphatase was 5.7 King-Armstrong units on the fifth postoperative day. On October 2, 1958, a right ureterolithotomy was performed, with an uncomplicated postoperative course. The patient was discharged from the hospital on October 11 feeling perfectly well, and has remained in good health since that time.

CASE II. Mrs. M. L. was first seen in 1940, at the age of twenty-two years, when she was admitted to the Maternity Division of the Royal Victoria Hospital for an incomplete abortion. At that time a firm tumor mass of the hard palate was noted. The patient stated that she had first noted this nodule about four months previously, and that it had been increasing in size and was becoming more tender. Eating was difficult because of pain in this mass. She also stated that for two years she had noticed a nodule in the right side of the neck, and for about a year a swelling on the dorsum of the left foot. She had lost about 40 pounds in the preceding year and also complained of transient bilateral swelling of the ankles and headaches associated with epistaxis.

Physical examination revealed a poorly nourished twenty-two year old woman feeling weak, but not appearing acutely ill. The temperature was 99°F., the pulse rate 96 per minute, the respiratory rate 24 per minute, and the blood pressure 148/85 mm. Hg. On the roof of the palate an oval shaped tumor about 5 by 2 cm. of hard rubbery consistency was noted; it was covered with thickened mucosa. A firm nodule was palpable in the right lobe of the thyroid. It was freely movable and moved with deglutition. The trachea was deviated to the left. A small palpable nodule was noted on the dorsum of the left foot in the region of the third metatarsal bone. Otherwise the physical examination was within normal limits.

Laboratory investigations included a urinalysis with a neutral reaction, specific gravity of 1.002, faint trace of albumin, no sugar and an occasional leukocyte on microscopic examination. The hemogram revealed a hemoglobin of 10.1 gm. per cent, a white blood cell count of 3,900 per cu. mm. and a sedimentation rate of 20 mm. per hour. The reaction to the blood Wassermann test was negative. The serum nonprotein nitrogen was 19.2 mg. per cent. Serum calcium levels were 14.5, 14.4 and 14.4 mg. per cent with serum inorganic phosphorus levels of 2.3 and 1.9 mg. per cent. The serum alkaline phosphatase measured 71, 51 and 83 King-Armstrong units.

Roentgenologic examination of the skull showed a diffuse demineralization and granularity of the bones of the cranial vault, and demineralization of the bones of the base of the skull. There was complete absence of all lamina dura of the teeth. The roentgenogram of the chest revealed a displacement of the cervical trachea to the left just above the thoracic inlet and this appeared to be due to a localized soft tissue mass extending for a distance of 2 cm. on the right side of the trachea. The thoracic spine appeared slightly demineralized, but the ribs did not appear abnormal. There was no evidence of active intrathoracic disease. The lumbar spine showed very little evidence of demineralization. The pelvis, however, had areas of cystic degeneration throughout the iliac wings on both sides, perhaps more marked on the left than on the right. Both humeri and both tibulas and fibulas showed fairly advanced demineralization and the medial aspects of upper ends of both tibias exhibited fairly extensive superiosteal erosion of bone. The left foot showed an area of cystic expansion of the proximal half of the third metatarsal; the cortex being almost completely destroyed in the proximal

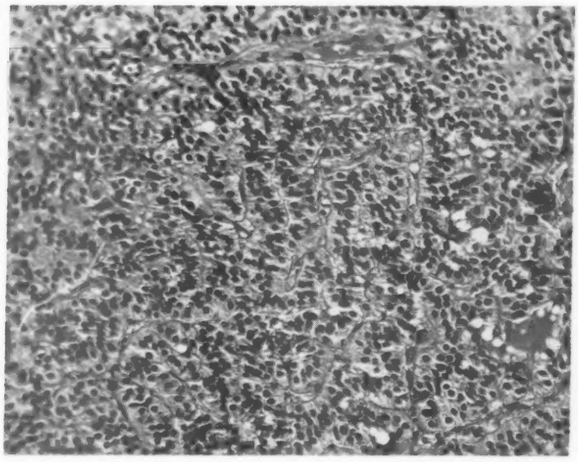

FIG. 3. Case II. Histologic section of parathyroid adenoma removed in 1940.

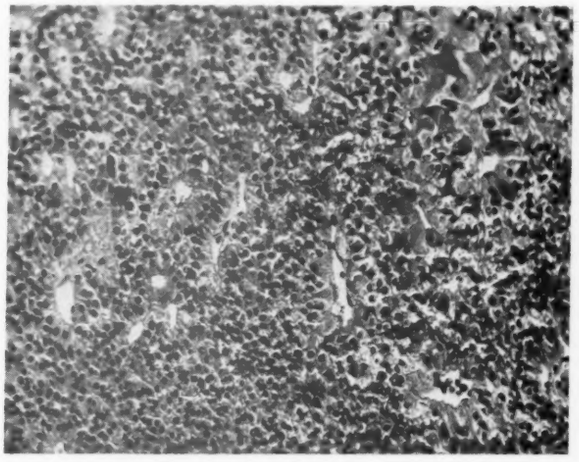

FIG. 4. Case II. Histologic section of parathyroid adenoma removed in 1958.

half. An intravenous pyelogram was unsuccessful, but the kidneys did not present gross evidence of nephrocalcinosis or nephrolithiasis.

On June 25, 1940, an exploratory operation of the neck revealed a large cystic mass about the size of a hen's egg pushing the right lobe of the thyroid forwards. The left lobe of the thyroid was normal. The cyst was removed along with part of the right lobe of the thyroid. On histologic section the tumor was composed of closely packed polyhedral-shaped cells with a round nucleus and rather pink cytoplasm. The cells tended to be arranged in strands and columns. (Fig. 3.) The biopsy specimen of the hard palate tumor revealed a giant cell tumor on histologic section.

Postoperatively, tetany developed, and the serum calcium level fell as low as 4.9 mg. per cent on the twenty-third postoperative day. The patient was treated with calcium gluconate, parathormone and AT-10, and the serum calcium levels rose to normal. On August 15, 1940, the patient suffered a pathologic fracture of the right femur, apparently at the site of a bone cyst. This was treated by traction in a Thomas splint, but the fracture failed to unite. Subsequently, on February 5, 1941, the fracture was treated with a Smith-Petersen pin and plate.

Postoperative roentgenologic examinations were made in order to follow the course of the disease. By July 1940 the cystic area in the proximal half of the third metatarsal showed definite early remineralization. By October 1940 this cyst was almost completely filled in by organized bone. By February 1941 the mineral content of the cranial vault had so markedly improved that the roentgenology appearance was almost normal. The density of the bones at the base of the skull had similarly improved, and lamina dura could be identified around several of the teeth in the mandible. By July 1941 the mineralization of the bones of the pelvis had returned to a normal appearance; the previously described cysts had been replaced by bone of normal density and trabecular pattern. The fracture of the right femur had completely united. Results of a skeletal survey in March 1946 were completely within normal limits.

The patient then felt well until 1951 when the presence of a right renal calculus led to hospitalization in another hospital where a nephrolithotomy was performed. She remained well until November 1957 when nervousness, emotional instability and vague aches and pains began to develop. She also became constipated; this increased in severity during the next few months. Beginning around March 1958 the patient noted polyuria and polydipsia, with nocturia three to four times. Muscle cramps, especially in the gastrocnemius group, became prominent and she began to experience vague bone pains in her legs, shoulder, spine and pelvic regions. She became anorexic and lost 27 pounds.

Investigations at her employment health agency revealed serum calcium levels of 13.8 and 13.5 mg. per cent, serum phosphorus of 3 and 2.2 mg. per cent and alkaline phosphatase 6.8 Bodansky units. Radioactive iodine uptake showed none over the right lobe of the thyroid, and a normal uptake over the left. A barium enema, barium meal, intravenous pyelogram and cholecystogram were within normal limits. Roentgenograms of the upper and lower extremities showed marked demineralization with evidence of slight subcortical bone resorption.

The patient was subsequently readmitted to the Royal Victoria Hospital in June 1958. Physical examination on admission revealed an emaciated forty-one year old white woman in no obvious distress, with a blood pressure of 110/75 mm. Hg, and a pulse rate of 76 per minute. There was a well healed transverse thyroidectomy scar and a suggestion of fullness in the right sternomastoid region, but no masses or nodules could be palpated. The lung fields were clear to auscultation and percussion. Examination of the cardiovascular system showed a grade 1 systolic murmur over the mitral and aortic

areas and along the left sternal border. There was some right upper quadrant tenderness on firm palpation of the abdomen. Central nervous system examination was normal and reflexes were symmetric and physiologic except for absence of ankle jerks. There was evidence of some loss of muscle mass but no definite evidence of weakness could be detected. The remainder of the physical examination was within normal limits.

Repeated urinalysis showed a specific gravity ranging between 1.007 and 1.013, a trace to 1-plus albumin, no sugar, a pH of 5.5 and an osmolarity of 259 mOsm. per L. Microscopic examination revealed moderate numbers of hyaline and finely granular casts, 5 to 10 white blood cells and 2 to 4 red cells. Reaction to urinary Sulkowitch test was 1-plus. The hemogram on admission showed hemoglobin of 11.9 gm. per cent, hematocrit of 37 per cent, sedimentation rate of 20 mm. per hour and white blood cell count of 11,400 per cu. mm. The serum nonprotein nitrogen was 29 mg. per cent. The serum calcium values were 14.5, 14.7 and 15.4 mg. per cent, with serum phosphorus levels of 2.2, 2.8 and 1.7 mg. per cent, alkaline phosphatase of 19.3, 13.1 and 14.6 King-Armstrong units. On diet containing 115 mg. of calcium per day the patient excreted 456, 410, 355 and 411 mg. of calcium per twenty-four hours. A tubular reabsorption of phosphorus on two occasions measured 77 and 73 per cent. Endogenous creatinine clearance was 79 cc. per minute.

Roentgenologic examinations of the skull, chest, thoracic and lumbar spines, abdomen, hands and pelvis revealed generalized demineralization which was more pronounced in the skull where the calvarium exhibited a speckled type of diffuse and fairly severe demineralization. Some of the vertebral bodies exhibited some concavity, but this was not marked. The dental areas examined showed a fairly marked, but not complete loss of lamina dura. A non-expanding cystic lesion was noted in the left ischial ramus, and several expanding cystic lesions were noted in the ribs, notably the fifth and eleventh on the right and the fifth, sixth and seventh on the left. The hands showed diffuse demineralization with a few areas of subperiosteal bone resorption in the middle phalanges. The mid-portion of the shaft of the distal phalanx of the third finger was completely destroyed by an expanding lesion, undoubtedly an expanding cyst. There was no evidence of nephrocalcinosis or nephrolithiasis.

On June 24, 1958, surgical exploration of the neck revealed a lobulated mass lying in close approximation to the right side wall of the trachea. The entire mass was resected except for a small portion of the posterior wall as the recurrent laryngeal nerve was adherent to it. On histologic section the tumor showed a richly vascular fibrous stroma containing a network of delicate capillary vessels which extended into the tumor and separated it into columns, sheets and alveoli formed of closely packed epithelial tumor cells. Cysts and areas of hemorrhage were not apparent. The tumor cells showed variation in nuclear size and staining affinity but no mitotic figures were seen. The tumor was composed of chief and oxyphilic cells, the chief cells predominating. (Fig. 4.)

Postoperatively a transient Chvostek's sign developed and the patient's course was complicated by bronchopneumonia. Otherwise the recovery was uneventful and the patient was discharged on July 11, 1958, with instructions to follow a high calcium diet, and to take calcium gluconate and vitamin D as directed. Since discharge the patient has remained well and periodic skeletal surveys have shown progressive remineralization of bone with healing of the bone cysts.

COMMENTS

Recurrence of hyperparathyroidism obviously may occur if an adenoma is not completely removed at the time of operation. Castleman [*4*] reported that three of six patients who had subtotal resection of the parathyroid adenoma (subtotal resections being performed to prevent postoperative hypocalcemia) later required a subsequent operation, at five months, seven years and fifteen years, but at the time of the second operation no further growth of the residuum was noted. Burk [*9*] described a patient with a parathyroid adenoma who came to surgery, but at the time of operation a piece of the adenoma was transplanted into the strap muscles of the neck in order to guard against postoperative hypocalcemia. A short while later there was recurrence of the hyperparathyroidism due to local growth of the transplanted tissue. Black and Ackerman [*10*] described a sixteen year old boy with hyperparathyroidism who was found to have a parathyroid adenoma in the lower pole of the right lobe of the thyroid. The capsule was ruptured at operation and the contents spilled into the area. The patient remained well until nine years later when he had a recurrence of hyperparathyroidism. At exploration a parathyroid adenoma was found at the upper pole of the right lobe of the thyroid, another mass at the site of the old operation, and on the anterior surface, four small nodules were observed.

There are other case reports, however, of recurrences of the adenoma that do not appear to be due to tissue accidentally or intentionally left behind. Shallow and Fry [*11*] reported a recurrence of a parathyroid adenoma fifteen years after the initial episode of hyperpara-

thyroidism. The patient, a thirty-five year old physician, was first seen in 1931 with symptoms of a gastrointestinal disturbance, polyuria, polydipsia, nervousness, irritability and shuffling gait starting four years previously, with weight loss. He also had multiple pathologic fractures. There was a palpable mass in the left lobe of the thyroid. The serum calcium level was 13 mg. per cent. A parathyroid adenoma was removed from the lower pole of the left lobe of the thyroid, with subsequent improvement so that he returned to work six months later. He remained well until 1946 when he was readmitted with recurrence of symptoms, hypercalcemia and bone changes. At operation a well encapsulated parathyroid adenoma was found on the right side. It is of interest that a parathyroid adenoma developed in his fourteen year old daughter in 1939; it was removed and there was an improvement in her condition over the next three months. A subsequent seven-year follow-up has not shown any recurrence. More recently, Greenfield, Verner and Engel [*12*] reported a case of recurrent adenoma, ten years after the initial removal. Hagtvet and Mathisen [*13*] described a case previously reported by Schrumpf and Harbitz [*14*]. At operation in 1936 two parathyroid adenomas were removed. At autopsy seven years later three other parathyroid adenomas were found. However, this case was complicated by renal disease and azotemia so that final diagnosis is difficult to evaluate.

At the Massachusetts General Hospital, Castleman [*15*] has had ten patients with double adenomas; in six they were removed at one operation. The other four had two operations. The first was a fifty-four year old woman who had a parathyroid adenoma removed from the left upper pole of the thyroid. Postoperatively the serum calcium level returned to normal. Five years later she had a recurrence, and at operation another small adenoma was found in the anterior mediastinum. The second patient had two adenomas removed nine years apart, one from the right upper pole of the thyroid and the other from the mediastinum. This patient, however, had no clinical response to the first operation. The third patient had only a mildly elevated serum calcium, but following removal of a left-sided adenoma the serum calcium level fell to the upper limits of normal. The urine, however, always contained markedly increased amounts of calcium, and within two years the serum calcium level was again elevated. Four years after the first operation a second operation was performed and a small adenoma was removed from the right side. This patient still had mild hyperparathyroidism nineteen years later, presumably due to the remaining portion of her adenoma. The fourth patient, a sixty-two year old white woman had excision of an adenoma following which the serum calcium and phosphorus levels returned to normal. She was seen seven years later when she was again found to have hyperparathyroidism and another parathyroid adenoma was removed from the mediastinum. Of these four patients it appears that two had both adenomas all along, and that in two another adenoma developed subsequent to the removal of the first. None of the second adenomas were in a location where they could be considered recurrences of part of the first adenoma accidentally left behind or disseminated at the first operation.

Of our two patients, it seems clear that in the first (Case I) a new adenoma developed following the first operation. She had felt well in the intervening years, the serum chemical determinations were normal, and the second adenoma was on the opposite side. On the other hand, in the second patient (Case II) both adenomas were on the same side. It is possible that recurrence was due to growth of tissue left behind, but she had been well for a long time after the first operation, and a skeletal survey as late as 1946 revealed no bony abnormalities.

SUMMARY

Two cases of recurrent hyperparathyroidism due to recurrent parathyroid adenomas are presented, with a review of the literature of similar cases.

Acknowledgment: We are indebted to Dr. R. G. Fraser for the review of the x-ray files on Mrs. M. L., and to Drs. B. Castleman and S. Roth for their permission to include their unpublished cases in our report.

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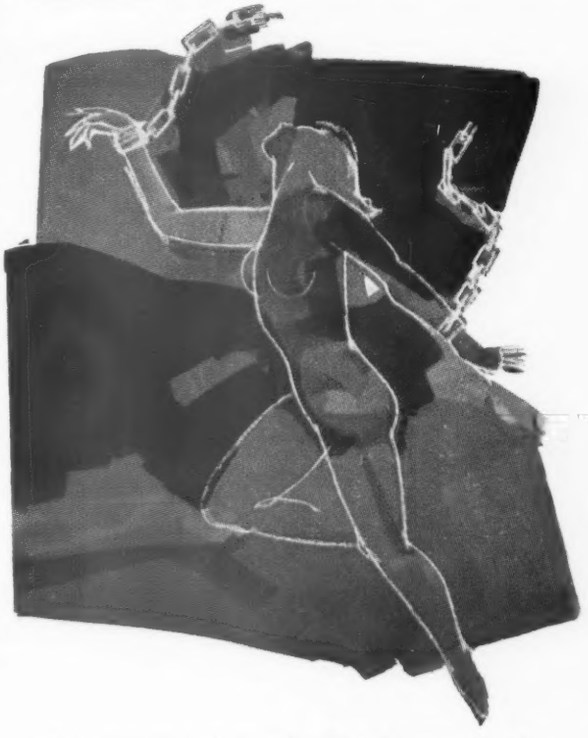

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*Warter, P. J.: Prednisolone-hydroxyzine combination in rheumatoid arthritis, J. M. Soc. New Jersey 54:7, 1957.

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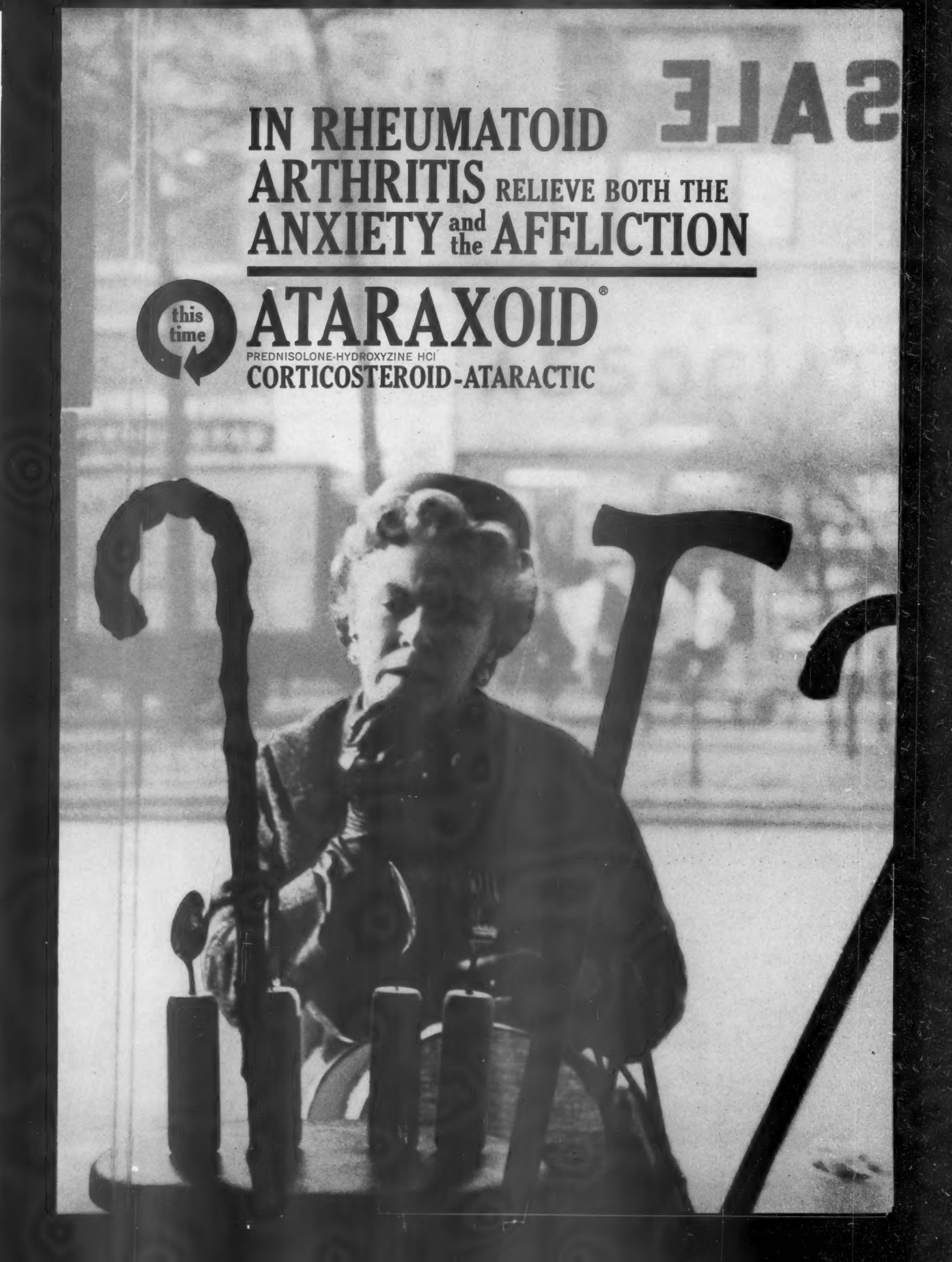

SALE
IN RHEUMATOID ARTHRITIS RELIEVE BOTH THE ANXIETY and the AFFLICTION
this time
ATARAXOID®
PREDNISOLONE-HYDROXYZINE HCl
CORTICOSTEROID-ATARACTIC

when your patient needs
a potent steroid... simplified control
of subacute or chronic disease...

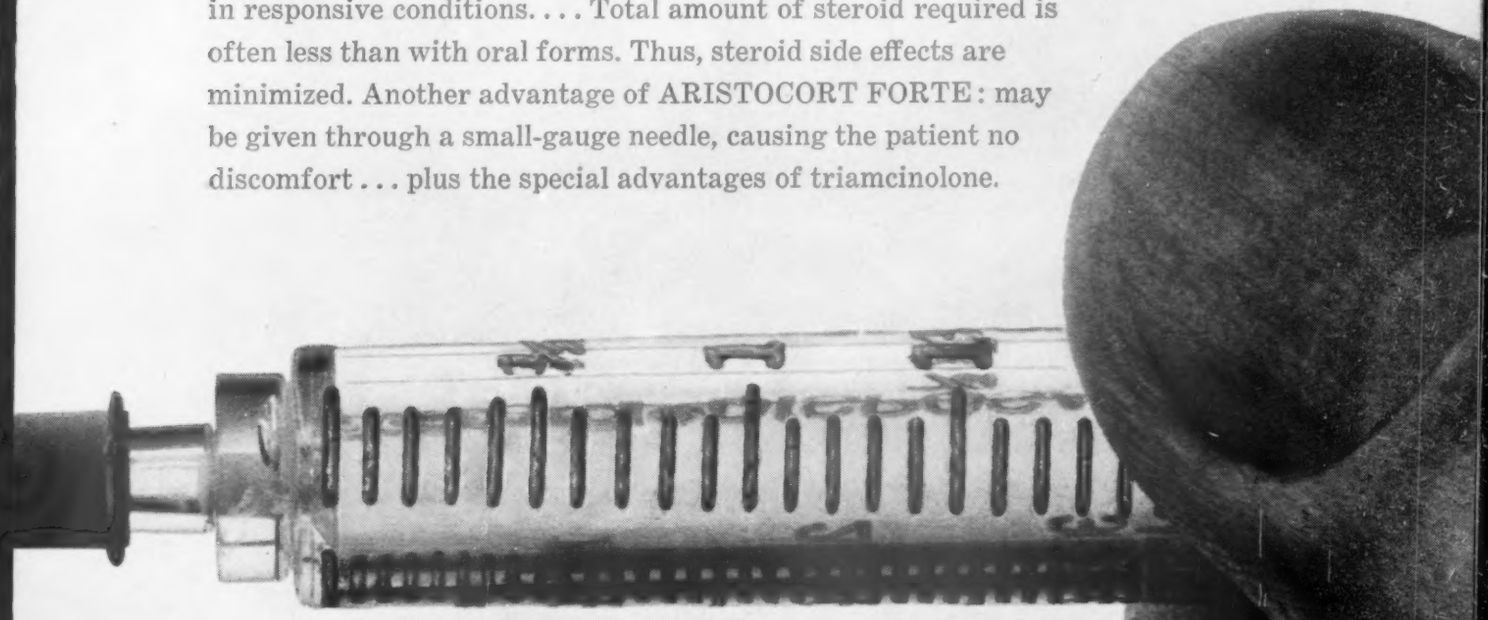
New Aristocort® Forte

TRIAMCINOLONE

Diacetate Parenteral Suspension Lederle

highly effective repository action with single, or infrequent, I.M. injections

Single I.M. doses of ARISTOCORT FORTE 4 to 7 times the usual daily oral dose can control symptoms 4 to 7 days, or even longer—sometimes up to 4 weeks in responsive conditions. . . . Total amount of steroid required is often less than with oral forms. Thus, steroid side effects are minimized. Another advantage of ARISTOCORT FORTE: may be given through a small-gauge needle, causing the patient no discomfort . . . plus the special advantages of triamcinolone.



INDICATIONS: Asthma and other allergies, including allergic rhinitis, hay fever, drug reactions; dermatoses, including psoriasis, poison ivy, urticaria, atopic eczema, pruritus; rheumatoid arthritis and other musculoskeletal conditions.

ARISTOCORT FORTE Parenteral – a suspension of 40 mg./cc. of triamcinolone diacetate micronized in: polysorbate 80 USP . . . 0.20%; polyethylene glycol 4,000 USP . . . 3%; sodium chloride . . . 0.85%; benzyl alcohol . . . 0.90%; water for injection q.s. . . . 100%; hydrochloric acid to approx. pH 6.

Not For Intravenous Use

Request complete information on indications, dosage, precautions and contraindications from your Lederle representative, or write to Medical Advisory Department.

Lederle LEDERLE LABORATORIES
A Division of AMERICAN CYANAMID COMPANY, Pearl River, New York

Renese®

POLYTHIAZIDE

1 mg. 2 mg. 4 mg.

A MORE CLINICALLY USEFUL
DIURETIC/ANTIHYPERTENSIVE

active antihypertensive
broad benefit
clinically confirmed
convenient control
dosage dexterity
dependable diuresis
enhanced effectiveness
foremost flexibility
increased individualization
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notable natruresis
orally optimal
peak potency
prolonged performance
reliable response
significant saluresis
tested toleration
unsurpassed utility
valuable versatility

"peak potency"—With intramuscularly administered meralluride serving as a standard value of 1.0 for potency comparison, human bio-assay has shown RENESE to be 2.1 times as potent as meralluride.[1]

1. Ford, R. V.: Current Therap. Res. 3:320, July, 1961.

Pfizer *Science for the world's well-being®*

PFIZER LABORATORIES Division,
Chas. Pfizer & Co., Inc. New York 17, New York

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IN PEPTIC ULCER AND HYPERACIDITY
with associated TENSION and NERVOUSNESS

NACTISOL*

- **suppresses gastric acid secretion at the parietal cell level**
- **decreases gastrointestinal hypermotility**
- **relieves nervousness and tension**

NACTISOL combines:

NACTON® 4 mg. (poldine methylsulfate†) — new inhibitor of gastric acid secretion and hypermotility "... reduces the total output of gastric HCl by about 60%"[1]

plus

BUTISOL SODIUM® 15 mg. (butabarbital sodium) — "daytime sedative" with highest therapeutic index[2] (highly effective, minimal side effects); smooth, predictable sedation of *6 hours' duration*

- Side effects with NACTISOL therapy have been minimal.[3-5]

NACTISOL*... in scored, yellow tablets—bottles of 100

Typical gastric secretory gland.

a —chief cell (pepsin-producing).

b —parietal cell (acid-producing).

NACTISOL INHIBITS GASTRIC ACID SECRETION AT THE PARIETAL CELL LEVEL

References

1. Douthwaite, A. H.: The Development of the Treatment of Duodenal Ulcer, Proc. Roy. Soc. Med. *51*:1063-1068 (December) 1958.
2. Batterman, R. C., Grossman, A. J., Leifer, P., and Mouratoff, G. J.: Clinical Re-evaluation of Daytime Sedatives, Postgrad. Med. *26*:502-509 (October) 1959.
3. Steigmann, F.: Clinical Report to McNeil Laboratories.
4. Lorber, S. H.: Clinical Report to McNeil Laboratories, December 6, 1960.
5. Rider, J. A.: Clinical Report to McNeil Laboratories.

McNEIL McNEIL LABORATORIES, INC., Fort Washington, Pa.

*Trademark

†U. S. Patent

Coming . . .

A SYMPOSIUM ON DIABETES

Topics that will be covered include:

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- ★ **The Microscopy and Chemistry of the Glomerulosclerotic Lesion**

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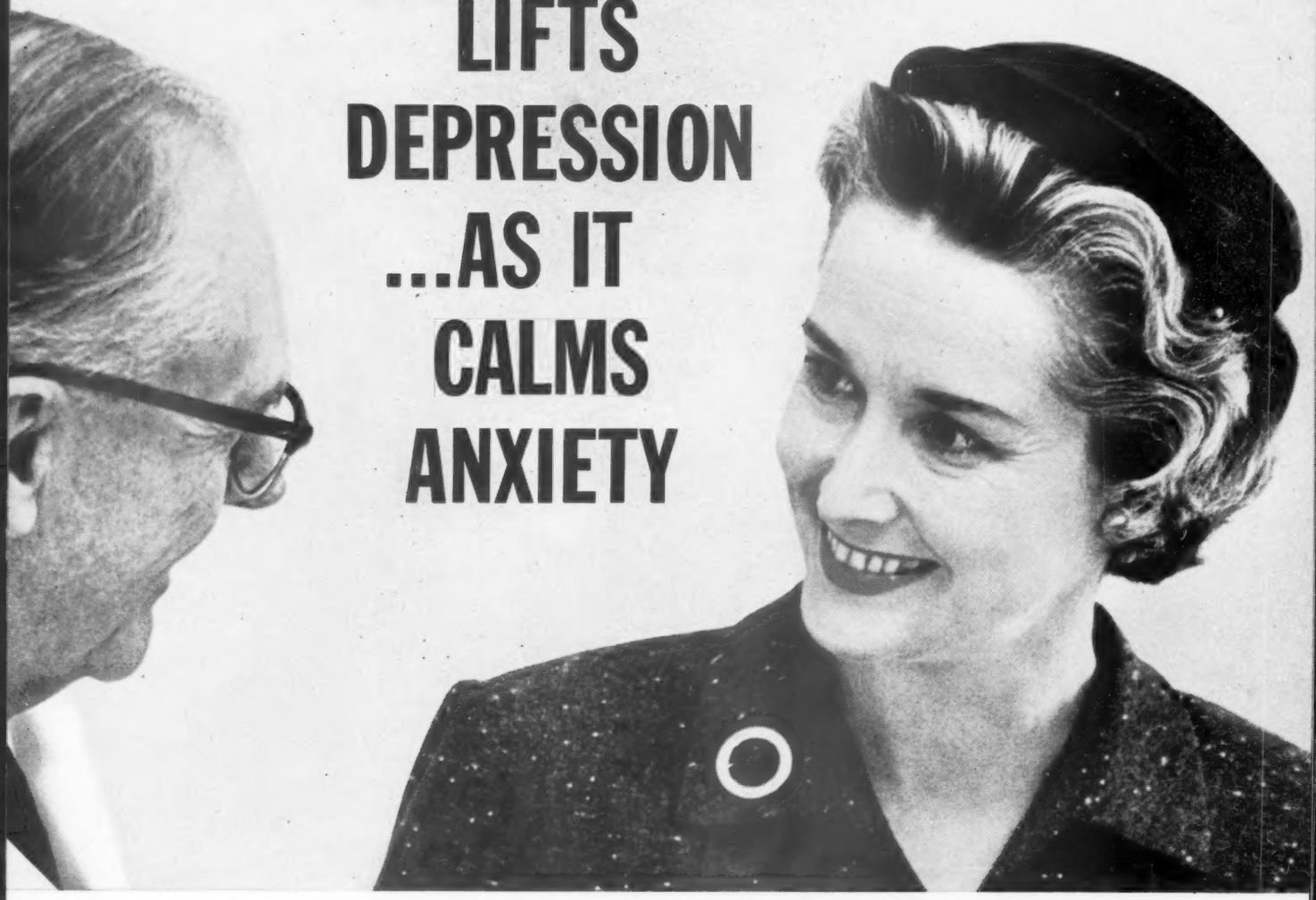
LIFTS DEPRESSION ...AS IT CALMS ANXIETY



"I feel like my old self again!" Thanks to your balanced Deprol therapy, normal drive and interest have replaced her emotional fatigue.

Brightens up the mood, brings down tension

Balanced action—avoids "seesaw" effects of energizers and amphetamines.

Acts rapidly—you see improvement in a few days.

Acts safely—no danger of liver or blood damage.

Dosage: Usual starting dose is 1 tablet q.i.d. When necessary, this dose may be gradually increased up to 3 tablets q.i.d.

Composition: 1 mg. 2-diethylaminoethyl benzilate hydrochloride (benactyzine HCl) and 400 mg. meprobamate.

Supplied: Bottles of 50 light-pink, scored tablets.

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Mail this coupon for clinical supply of Deprol

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Gentlemen: *Please send me a clinical supply of Deprol for the treatment of depression.*

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CONSIDER CITRUS PECTIN AND DERIVATIVES: *Pectin N.F., Pectin Cellulose Complex, Polygalacturonic and Galacturonic Acids*

Diarrheas, dysenteries—many other intestinal disorders—respond quickly and favorably to pharmaceutical specialties whose key ingredient is *an adequate dosage* of citrus pectin or a derivative.

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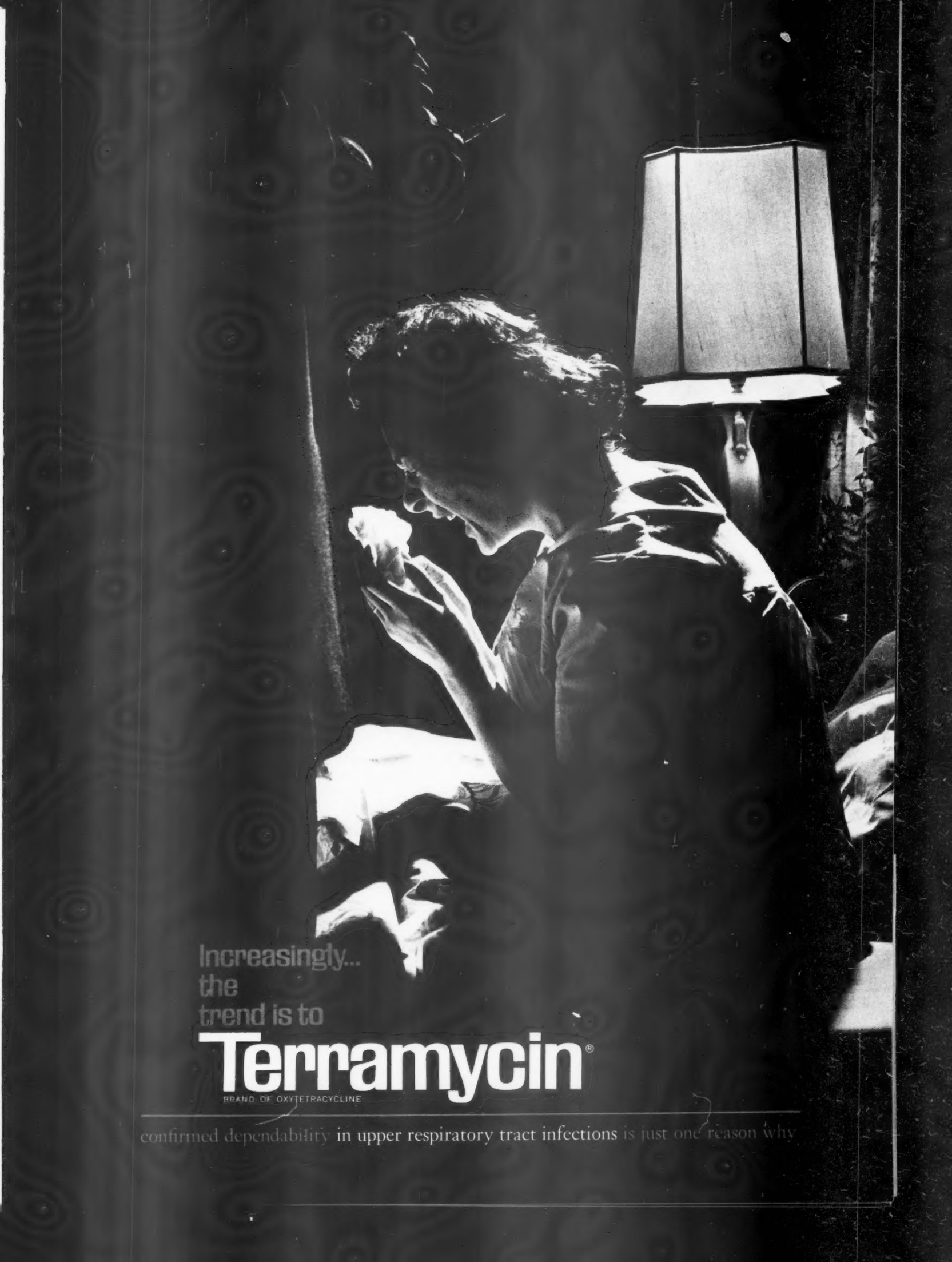
Increasingly...
the
trend is to
Terramycin®
BRAND OF OXYTETRACYCLINE
confirmed dependability in upper respiratory tract infections is just one reason why

Jacques and Fuchs* recently reported on experience with Terramycin in 106 patients with a variety of acute and chronic upper respiratory tract infections, many of which had failed to respond to other antibiotics. Oral dosage was preceded by intramuscular injection in 44 cases. "It was the impression of the hospital staff that oxytetracycline [Terramycin] was not only better tolerated, but more effective than other antibiotics habitually used."

The investigators noted that "some of the best results were seen in patients who were most severely ill."

According to the authors, the availability of Terramycin as a preconstituted intramuscular solution makes it feasible "to treat serious infections with a broad-spectrum antibiotic that has rapid action, and to continue with the same antibiotic given orally as soon as the infection is brought under control."

The results reported in this and many other studies confirm the vitality of Terramycin for broad-spectrum antibiotic therapy and demonstrate why—increasingly—the trend is to Terramycin.

In brief

The dependability of Terramycin in daily practice is based on its broad range of antimicrobial effectiveness, excellent toleration, and low order of toxicity. As with other broad-spectrum antibiotics, overgrowth of nonsusceptible organisms may develop. If this occurs, discontinue the medication and institute appropriate specific therapy as indicated by susceptibility testing. Glossitis and allergic reactions to Terramycin are rare. As with all I.M. preparations, injection should be made within the body of a relatively large muscle. Care should always be taken to avoid injection into a major nerve or its surrounding sheath. For complete dosage, administration, and precaution information, read package insert before using.

More detailed professional information available on request.

Terramycin®

BRAND OF OXYTETRACYCLINE

INTRAMUSCULAR SOLUTION

50 mg./cc. in 10 cc. vials; 100 mg. and 250 mg. in 2 cc. ampules

the broad-spectrum antibiotic ready for immediate intramuscular injection . . . conveniently preconstituted . . . notably well tolerated at injection site with low tissue reaction compared to other broad-spectrum antibiotics

another reason why the trend is to Terramycin—*versatility of dosage form:*

TERRAMYCIN Capsules
250 mg. and 125 mg. per capsule—for convenient initial or maintenance therapy in adults and older children

TERRAMYCIN Syrup / Pediatric Drops
125 mg. per tsp. and 5 mg. per drop (100 mg./cc.), respectively—deliciously fruit-flavored, preconstituted aqueous suspensions

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PFIZER LABORATORIES Division, Chas. Pfizer & Co., Inc.
New York 17, N. Y.

*Jacques, A. A., and Fuchs, V. H.: J. Louisiana M. Soc 113:200, May, 1961.

Pfizer *Science for the world's well-being®*

Dear Doctor:

Reports from our representatives indicate that many physicians would appreciate simplification for prescription-writing purposes of the names of Terramycin products in both the "plain" and the "Cosa" dosage forms.

The "Cosa" forms originated, you may recall, on the basis of clinical evidence of enhanced antibiotic absorption when glucosamine is employed in oral administration. To permit each physician individually to study this evidence and choose which form he would prefer to prescribe, we offered Terramycin in both forms—that is, in the regular Terramycin forms without glucosamine, and in the "Cosa" forms with glucosamine.

This distinction appears to be no longer necessary since glucosamine, a highly acceptable excipient for oral antibiotics, now is being incorporated uniformly in all such forms, thereby simplifying nomenclature and your prescription writing.

Accordingly, and effective immediately, forms incorporating glucosamine will be offered simply as Terramycin without the "Cosa" prefix.

To make clear just which forms are affected, please refer to the brief tabulation (below) of Terramycin dosage forms both *before* and *after* this change. We are also requesting our representative to call on you at an early date to answer any questions that may arise.

We feel certain that this action, prompted by your comments and those of many other physicians, will simplify your writing of prescriptions for Terramycin products.

We welcome your comments on this action and on any other phase of our operations, since it is our objective to render every service as efficiently as possible to our friends in the medical profession.

Sincerely,
PFIZER LABORATORIES

The following table indicates the former name and the current name of Terramycin systemic preparations:

FORMERLY NAMED	NOW NAMED
Cosa-Terramycin® Capsules	**Terramycin**® Capsules*
Cosa-Terrabon® Oral Suspension	**Terramycin** Syrup
Cosa-Terrabon Pediatric Drops	**Terramycin** Pediatric Drops

and simpler names for these Terramycin-containing formulations:

Cosa-Terrastatin® Capsules	**Terrastatin**® Capsules
Cosa-Terrastatin for Oral Suspension	**Terrastatin** for Oral Suspension
Cosa-Terracydin® Capsules	**Terracydin**® Capsules

. . . and these names remain unchanged:
Terramycin Intramuscular Solution
Terramycin Intravenous

**Terramycin Capsules* without glucosamine *are no longer available.*

The clinical versatility of Terramycin is enhanced by its specialized dosage forms adapted to individual needs—*another reason for the trend to Terramycin.*

BEFORE

LYOVAC®

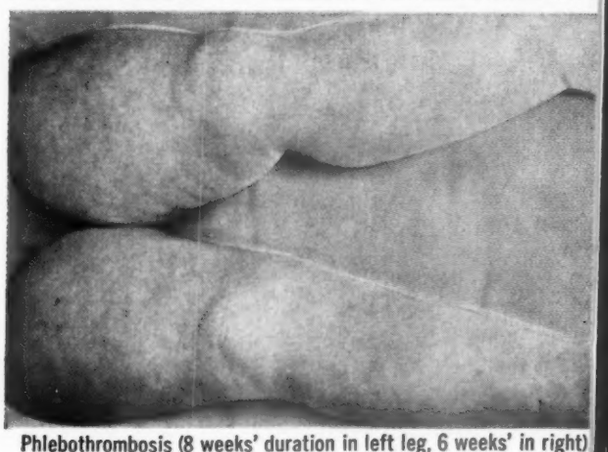
THROMBOLYSIN®

FIBRINOLYSIN (HUMAN)

a new agent
for lysis of
vascular thrombi

Phlebothrombosis of femoral and iliac vein, 3 weeks after onset.

Superior vena cava thrombosis (8 hrs. after starting THROMBOLYSIN).

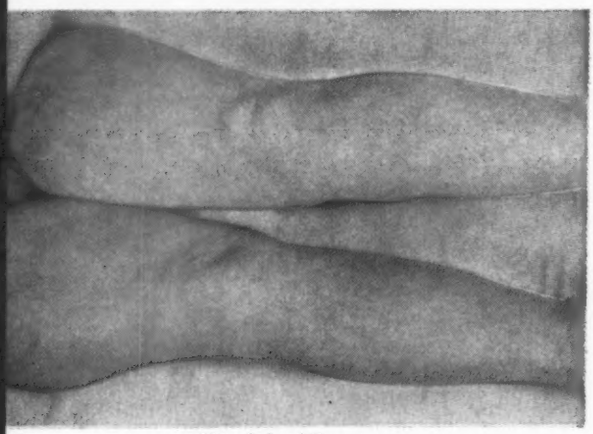


Phlebothrombosis (8 weeks' duration in left leg, 6 weeks' in right)

Thrombophlebitis of arm, 24 hours after onset.

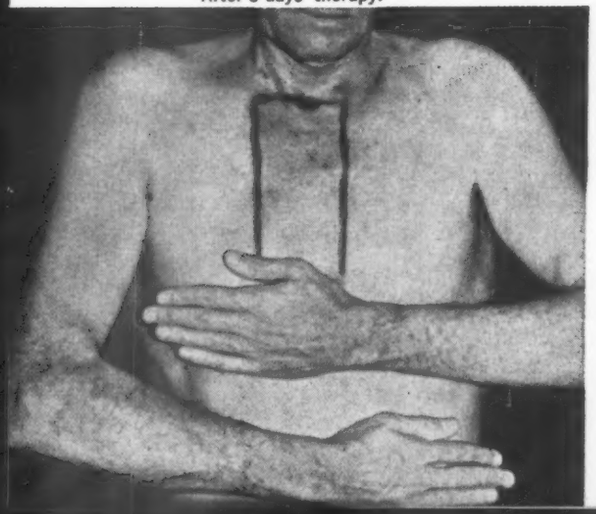
After 6 days' therapy.

After 3 days' therapy.



After 6 days' therapy.

After 5 days' therapy.



AFTER

LYOVAC®

THROMBOLYSIN®

FIBRINOLYSIN (HUMAN)

in thrombophlebitis, phlebothrombosis, pulmonary embolism, and certain arterial thrombi*, LYOVAC® THROMBOLYSIN makes possible

- lysis of formed clots
- reduced mortality and morbidity, shortened hospitalization
- reduced incidence of postphlebitic complications

with decreased incidence of reactions such as fever, chills, or malaise of severe proportions; higher degree of safety; greater, more predictable potency.

Supply: Each bottle contains 50,000 MSD units.

*See package circular for qualifications concerning cerebrovascular accidents and myocardial infarction.

Before prescribing or administering THROMBOLYSIN, *the physician should consult the detailed information on use accompanying the package or available on request.*

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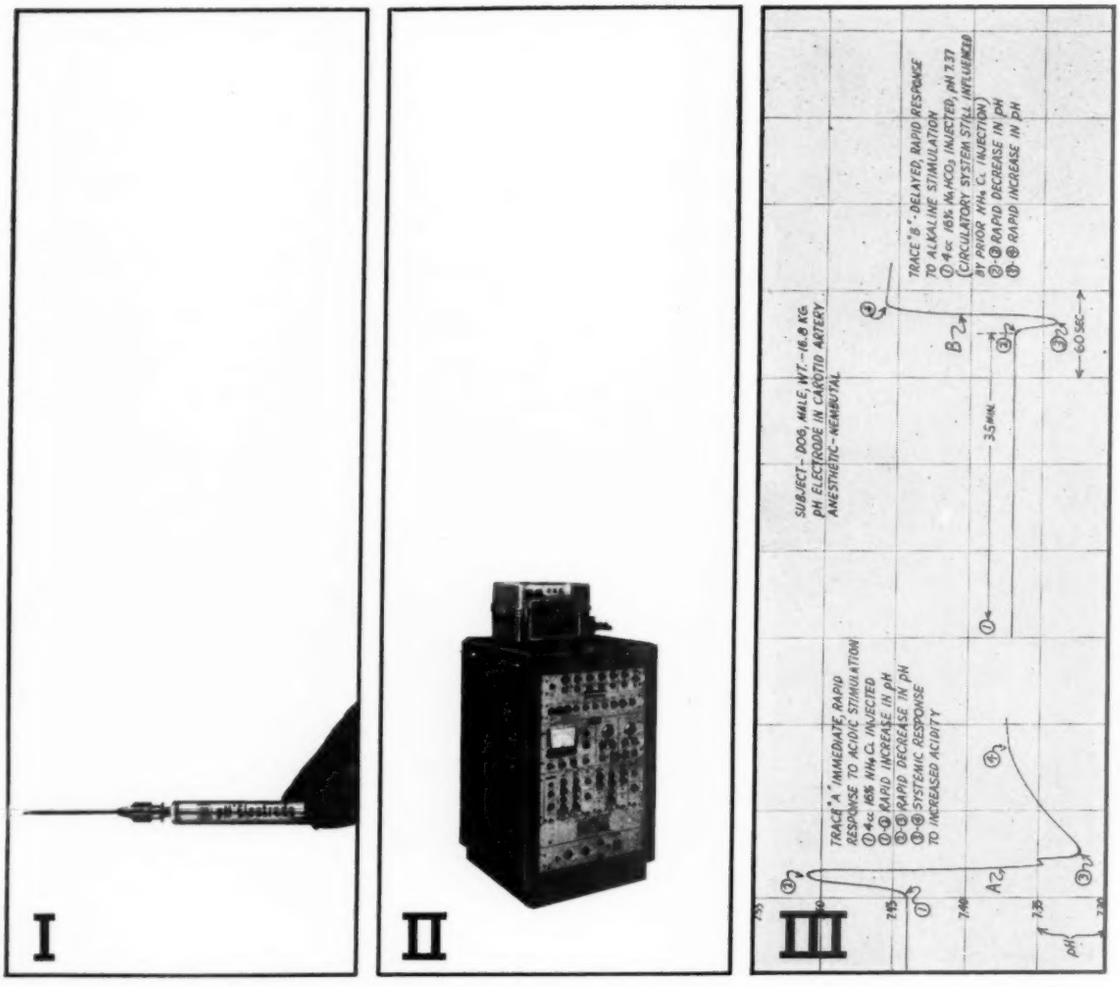

HONEYWELL MEASURES *in vivo* pH PLUS

Now being used in bio-medical research, the *in vivo* pH channel is one of the twenty-eight physiological measurement channels which you may select for your Honeywell Electronic Medical System.

The new Honeywell pH electrode enables you to now measure and record blood pH continuously with consistent accuracy. A one-piece instrument in which both active and reference electrodes are within a hypodermic needle, its accompanying circuitry is permanently sealed within the syringe barrel. The electrode (I) and its amplifier make up only one channel of a typical Honeywell Electronic Medical System (II).

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pH study courtesy T. W. Nielsen, Ph.D., Department of Physiology, University of Colorado Medical School

Low-back patient and muscle back in action

Prompt relief... early recovery—In low-back cases, or for any patient with inflammatory or traumatic musculoskeletal complaints, RELA offers prompt relief and the assurance of early recovery. In a study[1] of 212 conservatively treated low-back patients, 106 treated also with carisoprodol [RELA] were 'back in action' in one-fourth the time it took the conventionally treated group. RELA speeds recovery by a combination of effects—analgesic and muscle relaxant—to reduce spasm and tension, relieve pain, restore mobility. Undesirable effects have been minimal.

SUPPLIED: Bottles of 30, 350 mg. tablets. **REFERENCE:** 1. Kestler, O. C.: *J.A.M.A. 172*:2039 (April 30) 1960. For complete details, consult latest Schering literature available from your Schering Representative or the Medical Services Dept., Schering Corporation, Bloomfield, New Jersey. H-401

RELA®
carisoprodol
Schering

Protects the angina patient better than vasodilators alone

Unless the coronary patient's ever-present anxiety about his condition can be controlled, it can easily induce an anginal attack or, in cases of myocardial infarction, can delay recovery.

This is why Miltrate gives better protection for the heart than vasodilators alone in coronary insufficiency, angina pectoris and postmyocardial infarction.

Miltrate contains PETN (pentaerythritol tetranitrate), acknowledged as basic therapy for long-acting vasodilation. . . .

What is more important—Miltrate provides Miltown, a tranquilizer which, unlike phenobarbital, relieves tension in the apprehensive angina patient without inducing daytime fogginess.

Thus, your patient's cardiac reserve is protected against his fear and concern about his condition; his operative arteries are dilated to enhance myocardial blood supply—and he can carry on normal activities more effectively since his mental acuity is unimpaired by barbiturates.

REFERENCES: **1.** Ellis, L. B. *et al.:* Circulation *17*:945, May 1958. **2.** Friedlander, H. S.: Am. J. Cardiol. *1*:395, Mar. 1958. **3.** Riseman, J.E.F.: New England J. Med. *261*:1017, Nov. 12, 1959. **4.** Russek, H. I. *et al.:* Circulation *12*:169, Aug. 1955. **5.** Russek, H. I.: Am. J. Cardiol. *3*:547, April 1959. **6.** Tortora, A. R.: Delaware M. J. *30*:298, Oct. 1958. **7.** Waldman, S. and Pelner, L.: Am. Pract. & Digest Treat. *8*:1075, July 1957.

Supplied: Bottles of 50 tablets. Each tablet contains 200 mg. Miltown and 10 mg. pentaerythritol tetranitrate.

Dosage: 1 or 2 tablets q.i.d. *before meals* and at bedtime, according to individual requirements.

CML-3619

Miltrate®

Miltown® (meprobamate) + PETN

WALLACE LABORATORIES / *Cranbury, N. J.*

contains everything most coughers need...

... in each 5 cc. (1 teaspoonful):

Dimetane 2 mg.
(Parabromdylamine [Brompheniramine] Maleate),
the antihistamine most likely to succeed.

Phenylephrine HCl 5 mg. and Phenylpropanolamine HCl 5 mg.,
two highly approved decongestants.

Glyceryl Guaiacolate 100 mg.,
the expectorant that works best –
increases respiratory tract fluid almost 200%.

IN DIMETANE EXPECTORANT-DC
added Codeine Phosphate 10 mg./5 cc.(exempt narcotic),
when additional cough suppressant action is needed.

for less frequent, more productive cough...

Dimetane® Expectorant
Dimetane® Expectorant-DC

Robins

Dimetane Expectorant with Codeine Phosphate

A. H. ROBINS CO., INC., RICHMOND 20, VIRGINIA

helps you "reach" the depressed office patient

NIAMID®

BRAND OF NIALAMIDE

provides remission of depression—smoothly, gradually, without "jarring" □ notably low incidence of serious complications or side effects □ convenience of once-a-day dosage

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restores
the will
to
improve

In Brief Niamid, brand of nialamide, is 1-(2-[benzylcarbamyl] ethyl)-2-isonicotinylhydrazine, a well-tolerated antidepressant that may correct or relieve depression on once-a-day dosage. **Indications:** Depressive syndromes of varying degrees of severity may be responsive to Niamid including: involutional melancholia, postpartum depression, depressed phase of manic-depressive reaction, senile depression, reactive depression, schizophrenic reaction with depressive component, psychoneurotic depression. ■ In neurotic or psychotic patients, Niamid may normalize or favorably modify aberrant or excessive reactions and symptoms of depression such as: phobias, guilt feelings, dejection, feeling of inadequacy, discouragement, worry, uneasiness, distrustfulness, hypochondriacal and nihilistic ideas, difficulty in concentration, insomnia, loss of energy or drive, indecision, hopelessness, helplessness, decreased functional activity, emotional and physical fatigue, irritableness, inability to rest or relax, sadness, anorexia and weight loss, and withdrawal from society. *In the withdrawn patient,* Niamid may elevate the mood so that there is increased activity, increased awareness and interest in surroundings, and increased participation in group activities. Appetite may be increased and there may be decreased fatigability. Lack of clinical response to other antidepressant therapy does not preclude a favorable response to Niamid. Relief of depression may also be evidenced by elimination or reduction of the need for somatic therapy, such as electroshock. *In patients suffering from depression associated with chronic illness,* Niamid may improve mental outlook, reduce the impact of pain, decrease the amounts of narcotics or analgesics needed, and improve appetite and well-being. *In patients with angina pectoris,* Niamid has been found to be a useful adjunct to management through reduction in frequency of attacks and pain. **Dosage:** Starting dosage is 75 to 100 mg. on a once-a-day or divided daily basis. This may subsequently be adjusted depending upon the tolerance and response. Responses to Niamid are not usually rapid, and revisions of dose should be withheld until at least a few days have elapsed at each level. Increments or decrements of 12½-25 mg. are generally sufficient. A daily dosage of 200 mg. is the maximum recommended for routine use. (As much as 450 mg. daily has been used in some patients.) **Side Effects:** Niamid, in clinical use, has been characterized by a significant lack of toxicity. It is generally well tolerated. Nervousness, restlessness, insomnia, hypomania, or mania, sometimes occur. Occasional headache, weakness, lethargy, vertigo, dryness of the mouth, blurred vision, increased perspiration, constipation, mild skin rash, mild leukopenia, and epigastric distress may be obviated or modified by reductions in dose. Effects due to monoamine oxidase inhibition persist for a substantial period following discontinuation of the drug. **Precautions and Contraindications:** Hepatic toxicity has not been reported in extensive clinical studies. However, if previous or concurrent liver disease is suspected, the possibility of hepatic reactions and liver function studies should be considered. ■ The suicidal patient is always in danger, and great care must be exercised to maintain all security precautions. The apathetic patient may obtain sufficient energy to harm himself before his depression has been fully alleviated. ■ Niamid may potentiate sedatives, narcotics, hypnotics, analgesics, muscle relaxants, sympathomimetic agents, thiazide compounds and stimulants, including alcohol. Caution should be exercised when rauwolfia compounds and Niamid are administered simultaneously. Rare instances have been reported of reactions (including atropine-like effects, and muscular rigidity) occurring when imipramine was administered during or shortly after treatment with certain other drugs that inhibit monoamine oxidase. **In Cardiology:** The central effects of Niamid may encourage hyperactivity and the patient should be closely observed for any such manifestation. Orthostatic hypotension or hypertensive episodes occur in a few individuals; cardiac patients should be carefully selected and closely supervised. **In Epilepsy:** Although in some patients therapeutic benefits have been achieved with Niamid, in others the disease has been aggravated. Care should be exercised in the concomitant use of imipramine, since such treatment with monoamine oxidase inhibitors has been reported to aggravate the grand mal seizures. **In Tuberculosis:** Existing data do not indicate whether resistance of M. tuberculosis to isoniazid may be induced with Niamid therapy; nevertheless, it should be withheld in the depressed patient with coexisting tuberculosis who may need isoniazid. ■ As with all therapeutic agents excreted in part via the kidney, due caution in adjusting dosage in patients with impaired renal function should be observed. **Supplied:** Niamid (Nialamide) Tablets, 25 mg.: 100's—pink, scored tablets; 100 mg.: 100's—orange, scored tablets./*More detailed professional information available on request.*

Cremomycin® provides rapid relief of virtually all diarrheas

NEOMYCIN—actively bactericidal against a wide range of gram-negative intestinal pathogens, but relatively ineffective against certain diarrhea-causing organisms.

SULFASUXIDINE®, succinylsulfathiazole—an ideal adjunct to neomycin because it is highly effective against Clostridia and certain other neomycin-resistant organisms.

KAOLIN AND PECTIN—coat and soothe the inflamed mucosa, adsorb toxins, help provide rapid symptomatic relief.

Additional information on CREMOMYCIN is available to physicians on request.

MSD MERCK SHARP & DOHME, DIVISION OF MERCK & CO., INC., WEST POINT, PA.

CREMOMYCIN AND SULFASUXIDINE ARE TRADEMARKS OF MERCK & CO., INC.

"wearability"

355 cc. (12 Ounces)
List No. 346
MAALOX®
RORER
(MAGNESIUM-ALUMINUM HYDROXIDE GEL)
ANTACID — DEMULCENT
NON-CONSTIPATING
A colloidal suspension of Magnesium and Aluminum Hydroxides useful for the relief of gastric hyperacidity.
Shake Well Before Using
Average adult dose: As antacid and protective, two to four teaspoonfuls may be given in water or milk twenty minutes after meals and at bedtime. Use as directed by your physician. Maalox should not be used in patients who are severely debilitated or suffering from kidney failure.
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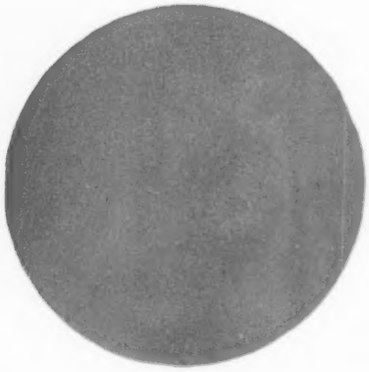

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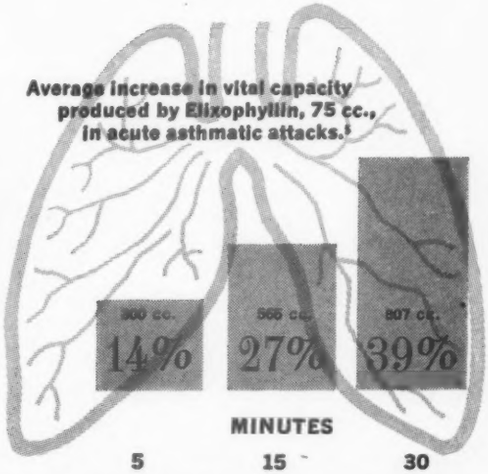

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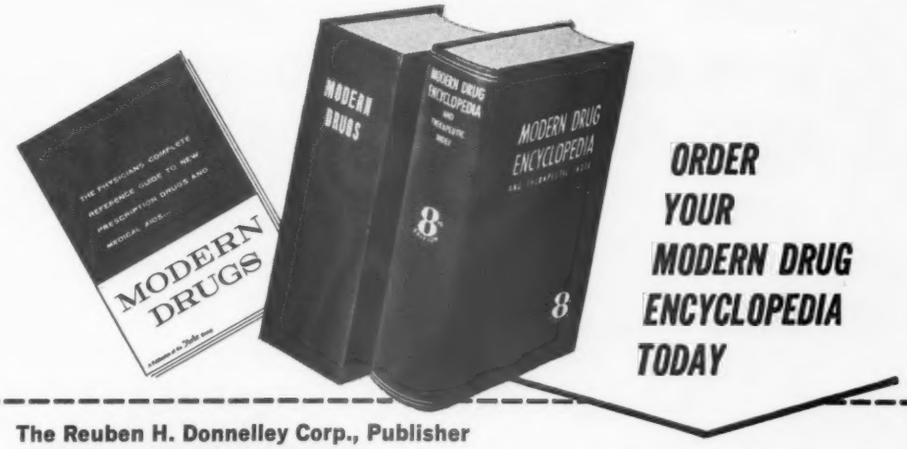

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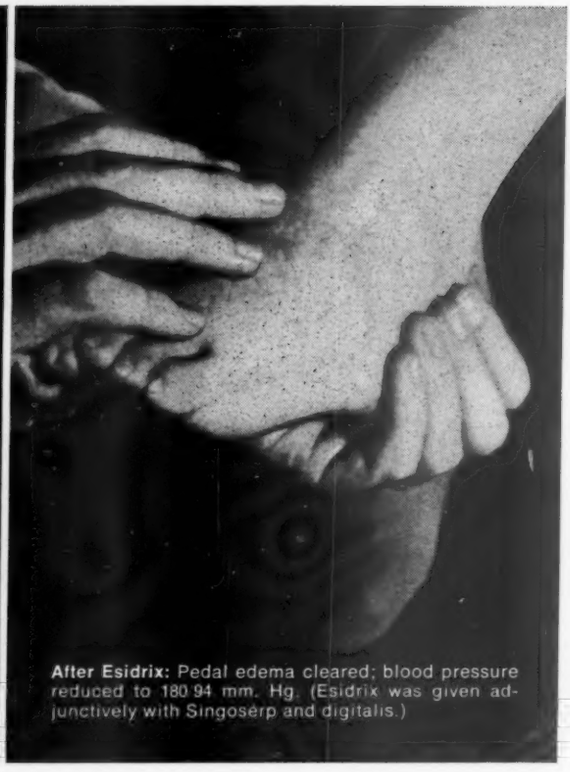

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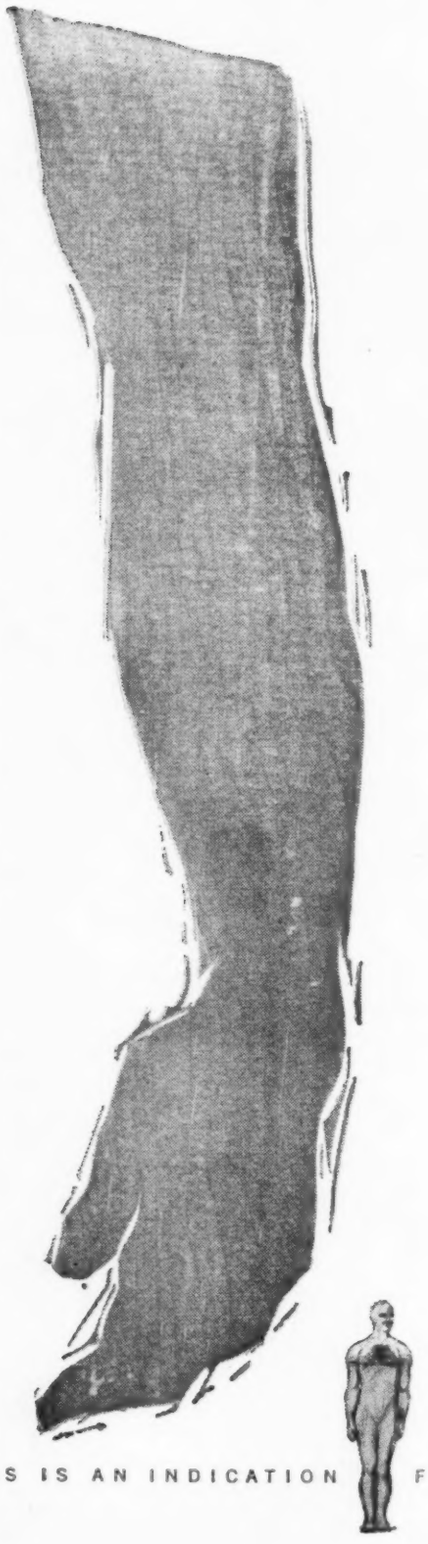

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REFERENCES: From the Symposium, *Recent Concepts of Pain and Analgesia*, held in the Hall of States, American Hospital Association, Chicago, February 15, 1961: 1. Batterman, R. C.: *Non-Narcotic Analgesia in Ambulatory Patients.* 2. O'Dell, T. B.: *Experimental Parameters in the Evaluation of Analgesics.* 3. Miller, L. D.: *Distribution, Excretion and Metabolic Fate of Phenyramidol.* 4. Beisler, E.: *Preliminary Report of Experience with Phenyramidol for Dental Analgesia.* 5. Bader, G.: *Preliminary Report on the Use of Analexin for Dysmenorrhea in Telephone Operators.* 6. Taylor, S. L.: *Phenyramidol in General Hospital Orthopedics.* 7. Bodi, T.: *Pain Management Among Clinic Outpatients.* 8. Ramunis, J.: *Experience of an Industrial Surgeon with Phenyramidol.* 9. Kast, E. C.: *Methodological Considerations in the Clinical Evaluation of an Analgesic.* 10. Collopy, C. T.: *Preliminary Comparisons of Two Non-Narcotic Analgesic Agents in Hospitalized Orthopedic Patients.* 11. Cass, L. J.: *Report on the Analgesic and Calmative Effectiveness of Two Preparations on Patients with Acute and Chronic Pain.* 12. Lamphier, T. A.: *Intravenous Phenyramidol in the Management of Low Back Pain and Allied Disorders.* 13. O'Dell, T. B.: Chicago Med. *63*:9, 1961. 14. Kast, E. C.: Chicago Med. *63*:17, 1961. 15. Wainer, A. S.: J. Am. M. Women's A. *16*:218, 1961. 16. Batterman, R. C.: Ann. New York Acad. Sc. *86*:203, 1960. 17. O'Dell, T. B.: Ann. New York Acad. Sc. *86*:191, 1960. 18. O'Dell, T. B., *et al.:* J. Pharmacol. & Exper. Therap. *128*:65, 1960. 19. O'Dell, T. B., *et al.:* Fed. Proc. *18*:1694, 1959. 20. Gray, A. P., *et al.:* J. Am. Chem. Soc. *81*:4347, 1959. 21. Wainer, A. S.: Clin. Med. *7*:2331, 1960. 22. Clinical data in files of Medical Dept., Irwin, Neisler & Co., 1959. 23. Batterman, R. C., *et al.:* Am. J. Med. Sc. *238*:315, 1959.

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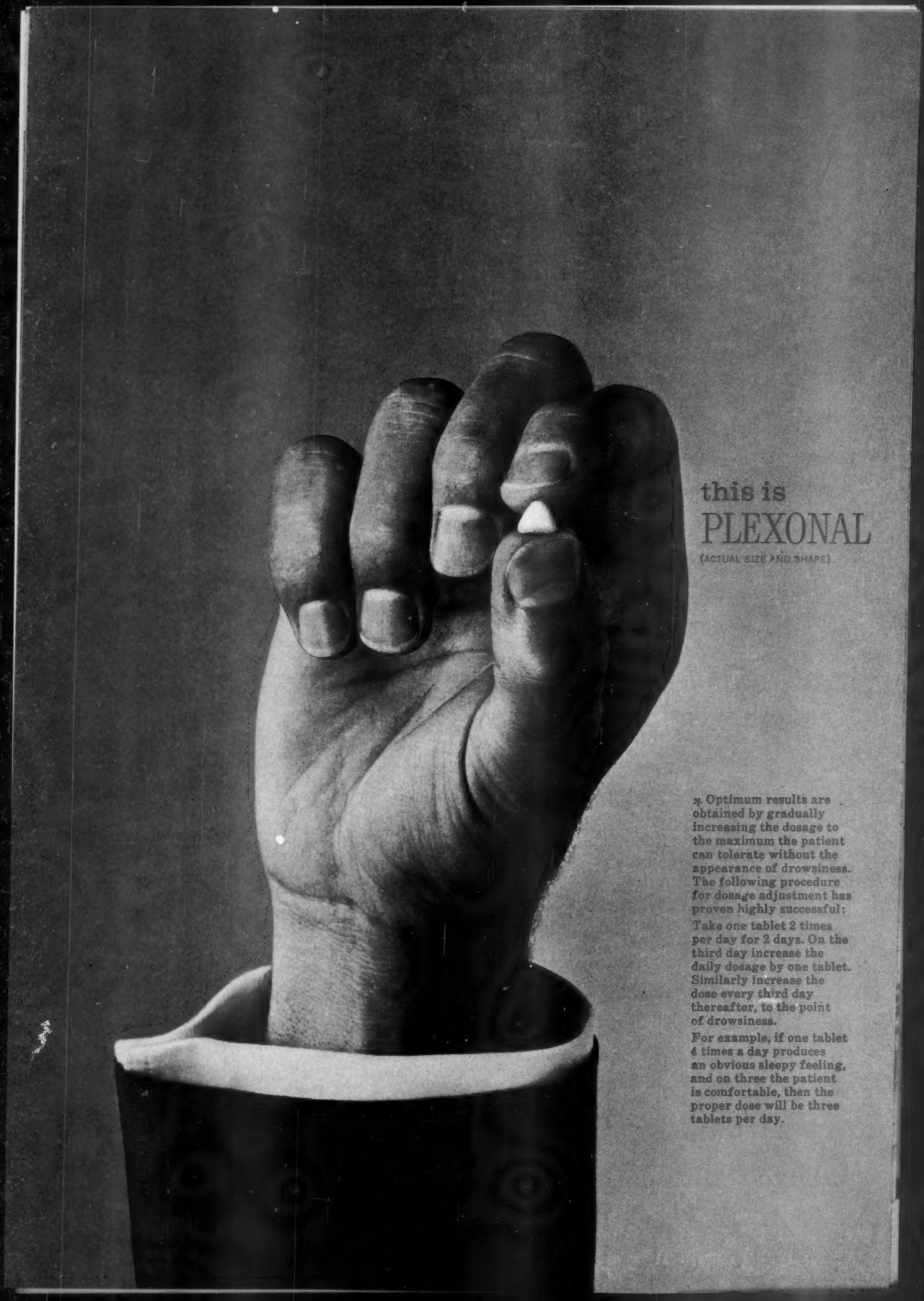

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Extremely well tolerated by geriatric patients who need mild sedation, as well as by depressed patients.

Dosage: One tablet 3 or 4 times a day is adequate for most patients. However, some require up to six tablets per day, whereas others respond adequately to as little as 1 tablet per day.

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1. Scheifley, C. H.: Proc. Staff Meet. Mayo Clin. 34:408 (Aug. 19) 1959.
2. Davanloo, H.: Am. J. of Psychiat. 117:740 (Feb.) 1961.

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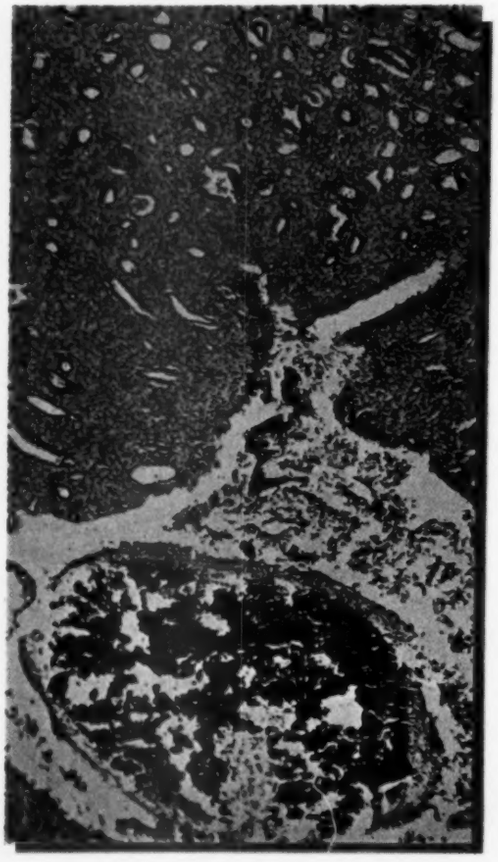
GOUT

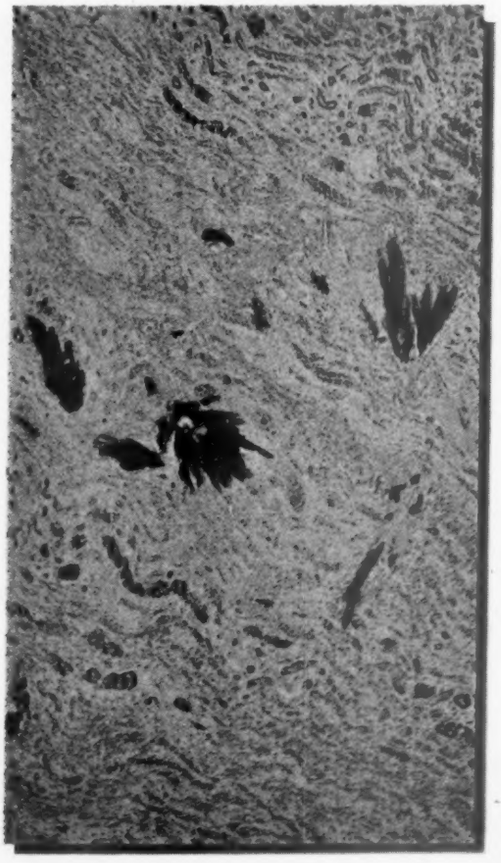
"For centuries the victims of gout have been the subject of lampoons and caricatures. We now know that they should rather be the objects of great concern, for the disease is painful, disabling and is accompanied by complications that impair health and shorten life."[1]

Kidney impairment, with varying degrees of hypertension and arteriosclerosis, is the critical complication of gout. "From 30 to 50 per cent of gouty patients are said to die of renal disease."[2]

Tophus in the calyx, surrounded by fibrinoid exudate and detached mucosal epithelium.[3]



The mid- and outer portion of the pyramid with typical uric acid crystals in the collecting system.[3]



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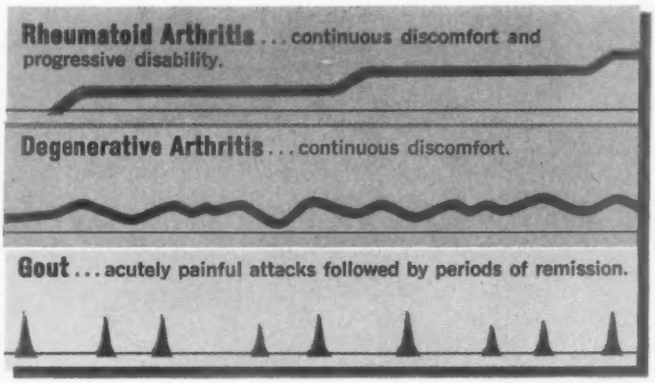
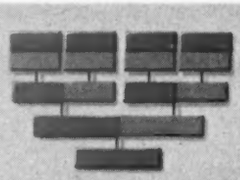
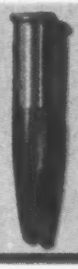

Rheumatoid Arthritis . . . continuous discomfort and progressive disability.
Degenerative Arthritis . . . continuous discomfort.
Gout . . . acutely painful attacks followed by periods of remission.



"If a family history of gout is obtained, even though it is one or two generations removed, this information is significant."[4]

"The metatarso-phalangeal joint of a great toe is affected early or repeatedly in some cases, rarely or never in others."[6]



"All patients complaining of non-traumatic musculoskeletal discomfort should have at least one serum uric acid determination."[5]

Colchicine test: "Colchicine should be administered as early as possible after the onset of articular distress, 1 mg. . . . every 2 hours until the onset of gastro-intestinal distress. From 5 to 8 mg. usually are required."[9] Pain relief is highly indicative of gout.

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Dosage: One tablet daily for one week, followed by one tablet twice daily. Supply: Each tablet contains 0.5 mg. colchicine and 0.5 Gm. BENEMID. Bottles of 100 and 1000.

1. Cornish, A. L.: J. Kentucky M.A. 58:707, June, 1960. 2. Wyngaarden, J. B.: Arthritis & Rheumatism 1:191, June, 1958. 3. Talbott, J. H. and Terplan, K. L.: Medicine 39:405, Dec., 1960. 4. Talbott, J. H.: Gout, New York, Grune & Stratton, 1957, p. 123. 5. Kuzell, W. C., et al.: J. Chron. Dis. 2:645, Nov., 1955. 6. Hench, P. S.: Gout and gouty arthritis, in Cecil, R. L.: A textbook of medicine, ed. 10, Phila., W. B. Saunders Co., 1959. 7. Bartels, E. C., and Kepkay, P. H.: Bull. Vancouver M.A. 29:306, April, 1953. 8. Boland, E. W.: World-Wide Abstracts of Gen. Med. 3:16, Jan., 1960. 9. Talbott, J. H.: Current Med. Dig. 26:57, Nov., 1959.

Before prescribing or administering BENEMID or ColBENEMID, the physician should consult the detailed information on use accompanying the package or available on request.

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Ten years of world-wide experience... almost 2000 published reports... have progressively entrenched Butazolidin as the leading nonhormonal antiarthritic agent.

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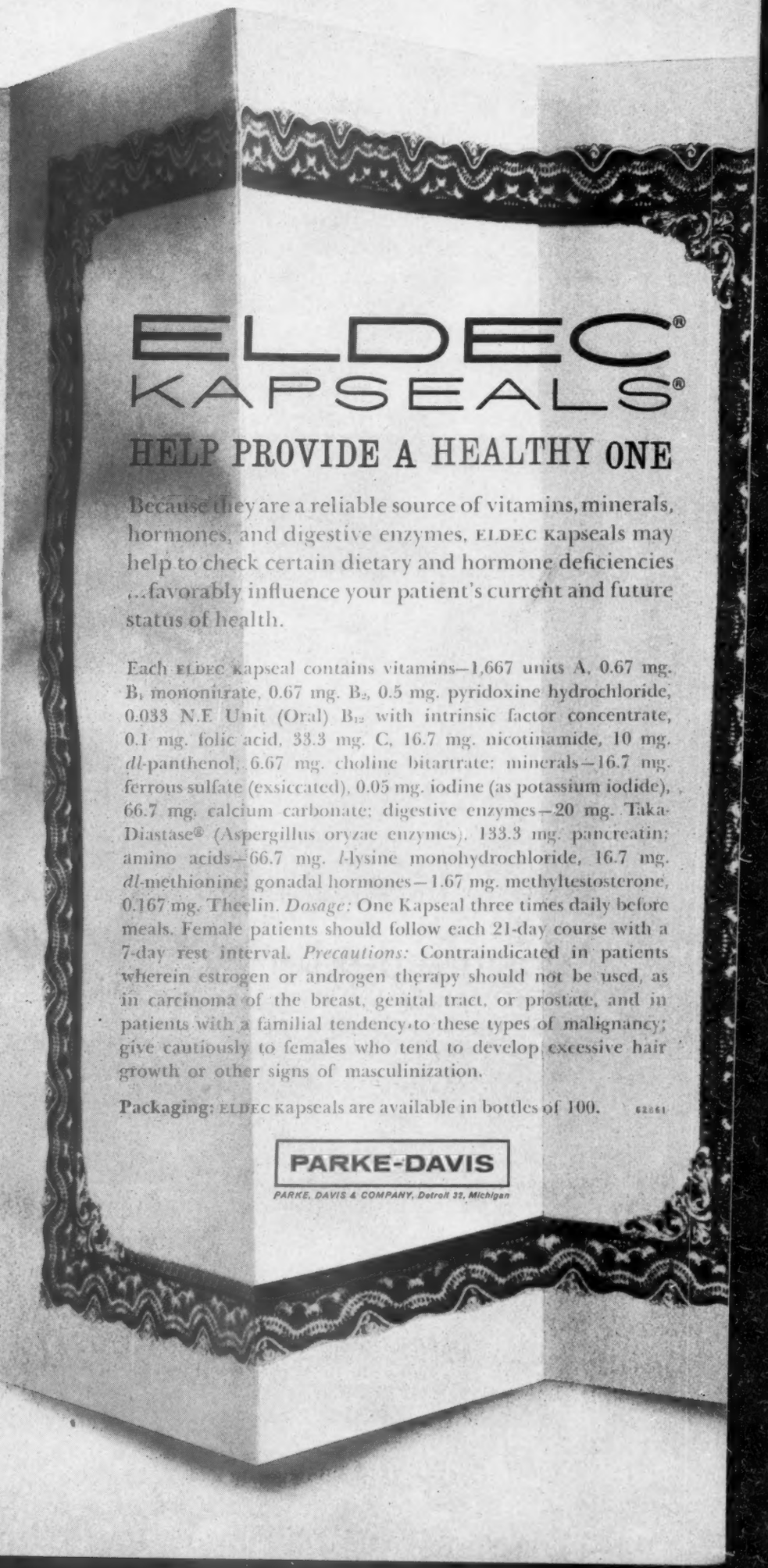

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Each ELDEC Kapseal contains vitamins—1,667 units A, 0.67 mg. B_1 mononitrate, 0.67 mg. B_2, 0.5 mg. pyridoxine hydrochloride, 0.033 N.F. Unit (Oral) B_{12} with intrinsic factor concentrate, 0.1 mg. folic acid, 33.3 mg. C, 16.7 mg. nicotinamide, 10 mg. *dl*-panthenol, 6.67 mg. choline bitartrate; minerals—16.7 mg. ferrous sulfate (exsiccated), 0.05 mg. iodine (as potassium iodide), 66.7 mg. calcium carbonate; digestive enzymes—20 mg. Taka-Diastase® (Aspergillus oryzae enzymes), 133.3 mg. pancreatin; amino acids—66.7 mg. *l*-lysine monohydrochloride, 16.7 mg. *dl*-methionine; gonadal hormones—1.67 mg. methyltestosterone, 0.167 mg. Theelin. *Dosage:* One Kapseal three times daily before meals. Female patients should follow each 21-day course with a 7-day rest interval. *Precautions:* Contraindicated in patients wherein estrogen or androgen therapy should not be used, as in carcinoma of the breast, genital tract, or prostate, and in patients with a familial tendency to these types of malignancy; give cautiously to females who tend to develop excessive hair growth or other signs of masculinization.

Packaging: ELDEC Kapseals are available in bottles of 100. 62861

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POLYTHIAZIDE

1 mg. 2 mg. 4 mg.

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DIURETIC/ANTIHYPERTENSIVE

active antihypertensive
broad benefit
clinically confirmed
convenient control
dosage dexterity
dependable diuresis
enhanced effectiveness
foremost flexibility
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1. Ford, R. V.: Current Therap. Res. 3:320, July, 1961.

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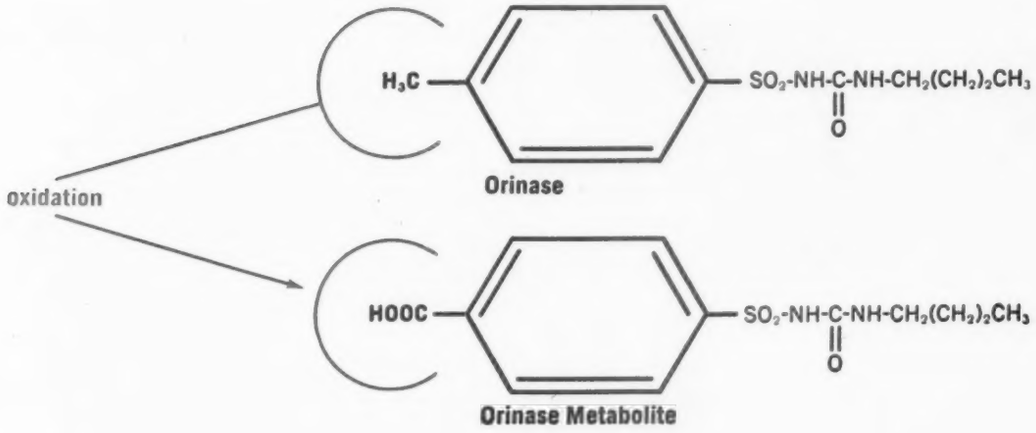
FOR PRODUCT INFORMATION TURN TO PAGE 12

Why is the methyl "governor" in Orinase so important?

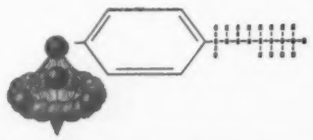
One of the most significant advantages of Orinase therapy is the rarity of associated hypoglycemic reactions.

This widely-reported clinical benefit is a function of the exclusive Orinase methyl "governor." Lending itself to ready oxidation (principally, it is thought, a hepatic process), the methyl group ensures prompt metabolic inactivation of the Orinase molecule. What actually happens is that a rapidly- and continuously-excreted carboxy-metabolite is produced that has no hypoglycemic activity at the existing levels.

As a result of the oxidation of its methyl group, Orinase shows a decline in activity soon after it reaches its effective peak in the plasma. Maintenance dosage serves to reduce blood sugar levels to normal, but rarely below that point, and there is no reported problem of accumulation.



Orinase*



An exclusive methyl "governor" minimizes hypoglycemia

Indications and effects: The clinical indication for Orinase is stable diabetes mellitus. Its use brings about the lowering of blood sugar; glycosuria diminishes, and such symptoms as pruritus, polyuria, and polyphagia disappear.

Dosage: There is no fixed regimen for initiating Orinase therapy. A simple and effective method is as follows: *First day*—6 tablets; *second day*—4 tablets; *third day*—2 tablets. The daily dose is then adjusted—raised, lowered or maintained at the two-tablet level, whichever is necessary to maintain optimum control.

Patients receiving insulin (less than 20 units)—discontinue insulin and institute Orinase; (20 to 40 units)—initiate Orinase with a concurrent 30 to 50% reduction in insulin dose with a further careful reduction as response to Orinase is observed; (more than 40 units)—reduce insulin by 20% and initiate Orinase with a further careful reduction in insulin dosage as response to Orinase is observed. In candidates for combined Orinase-insulin therapy, an individualized schedule is usually obtainable during a trial course of two or more weeks.

Contraindications and side effects: Orinase is contraindicated in patients having juvenile or growth-onset, unstable or brittle types of diabetes mellitus; history of diabetic coma, fever, severe trauma or gangrene.

Side effects are mild, transient and limited to approximately 3% of patients. Hypoglycemia and toxic reactions are extremely rare. Hypoglycemia is most likely to occur during the period of transition from insulin to Orinase. Other untoward reactions to Orinase are usually not of a serious nature and consist principally of gastrointestinal disturbances, headache, and variable allergic skin manifestations. The gastrointestinal disturbances (nausea, epigastric fullness, heartburn) and headache appear to be related to the size of the dose, and they frequently disappear when dosage is reduced to maintenance levels or the total daily dose is administered in divided portions after meals. The allergic skin manifestations (pruritus, erythema, and urticarial, morbilliform, or maculopapular eruptions) are transient reactions, which frequently disappear with continued drug administration. However, if the skin reactions persist, Orinase should be discontinued.

Clinical toxicity: Orinase appears to be remarkably free from gross clinical toxicity on the basis of experience accumulated during more than four years of clinical use. Crystalluria or other untoward effects on renal function have not been observed. Long-term studies of hepatic function in humans and experience in over 650,000 diabetics have shown Orinase to be remarkably free of hepatic toxicity. There has been reported only one case of cholestatic jaundice related to Orinase administration, which occurred in a patient with pre-existing liver disease and which rapidly reversed upon discontinuance of the drug.

Each tablet contains:
Tolbutamide ..... 0.5 Gm.

Supplied: In bottles of 50.

*Trademark, Reg. U.S. Pat. Off.—tolbutamide, Upjohn

June, 1961

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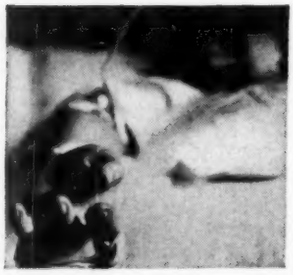
not a general-purpose antibiotic



Albamycin is not a broad-spectrum antibiotic, recommended for routine infections. It is specific for staphylococci (including resistant strains), and its use alone should (with the exceptions listed below) be limited to those cases in which staph is known or strongly suspected to be the causative organism.

Albamycin*

Indications—Albamycin is indicated in the treatment of staphylococcic infections, particularly in patients sensitive to other antibiotics or in the infections in which the organism is resistant to other antibiotics and sensitive to Albamycin, and in urinary tract infections due to microorganisms resistant to other commonly employed antibacterial agents but sensitive to Albamycin—notably certain strains of Proteus.

Administration and Dosage—Capsules and Syrup: The recommended dosage in adults is 500 mg. every twelve hours or 250 mg. every six hours, continued for at least forty-eight hours after the temperature has returned to normal and all evidence of infection has disappeared. In severe or unusually resistant infections, 0.5 Gm. every six hours or 1 Gm. every twelve hours may be employed. The dose for children is 15 mg. per kilogram of body weight per day for moderately acute infections; this may be increased to 30 to 45 mg. per kilogram of body weight per day for severe infections. These doses may be administered on schedules similar to those for adults.

Parenteral: Intramuscularly—5 cc. of Albamycin solution may be used directly by slow injection deep into the gluteal muscle.

Intravenously—It is recommended that 5 cc. of Albamycin solution be diluted further with 250 to 1000 cc. of sterile injection solution of sodium chloride, Darrow's solution, or Ringer's solution and administered by intravenous infusion, or by diluting to a suitable quantity and administered by continuous drip infusion. **Do not use with dextrose solution.** When it is necessary to use a smaller volume intravenously, 5 cc. of Albamycin solution may be diluted to a minimum of 30 cc. with one of the above diluents and administered slowly over a period of five to ten minutes to avoid irritation of the vascular endothelium. The dosage for adults is 500 mg. Albamycin administered either intramuscularly or intravenously every twelve hours. For children with moderately acute infections, the dosage is 15 mg. per kilogram of body weight per day. The daily dosage should be administered in two divided doses at intervals of twelve hours. As soon as the patient's condition permits, parenteral Albamycin should be replaced with oral Albamycin therapy.

Side Effects—Albamycin is a substance of low toxicity but is capable of inducing urticaria and maculopapular dermatitis. Leukopenia, which was rapidly reversible, has been reported in approximately 1% of cases. All of these side effects disappear rapidly upon discontinuance of the drug. In a certain few patients, a yellow pigment has been found in the plasma. This pigment is a metabolic by-product of the drug which, however, may interfere with determination of bilirubin and icteric index. Its presence is not associated with abnormal liver function tests or liver enlargement.

Available—Albamycin, 500 mg., sterile, Mix-O-Vial.† Each Mix-O-Vial contains: 500 mg. Novobiocin (as novobiocin sodium), also 175 mg. Nicotinamide; 0.47 cc. N,N-Dimethylacetamide; 42.3 mg. Benzyl alcohol; 4.23 cc. water for injection. Albamycin Capsules. Each capsule contains: 250 mg. Novobiocin (as novobiocin sodium). Albamycin Syrup. 125 mg. per 5 cc. Each 5 cc. (one teaspoonful) contains: 125 mg. Novobiocin (as novobiocin calcium). Preserved with methylparaben, 0.075%, and propylparaben, 0.025%.

*Trademark, Reg. U. S. Pat. Off.—The Upjohn brand of crystalline novobiocin sodium. †Trademark, Reg. U. S. Pat. Off.

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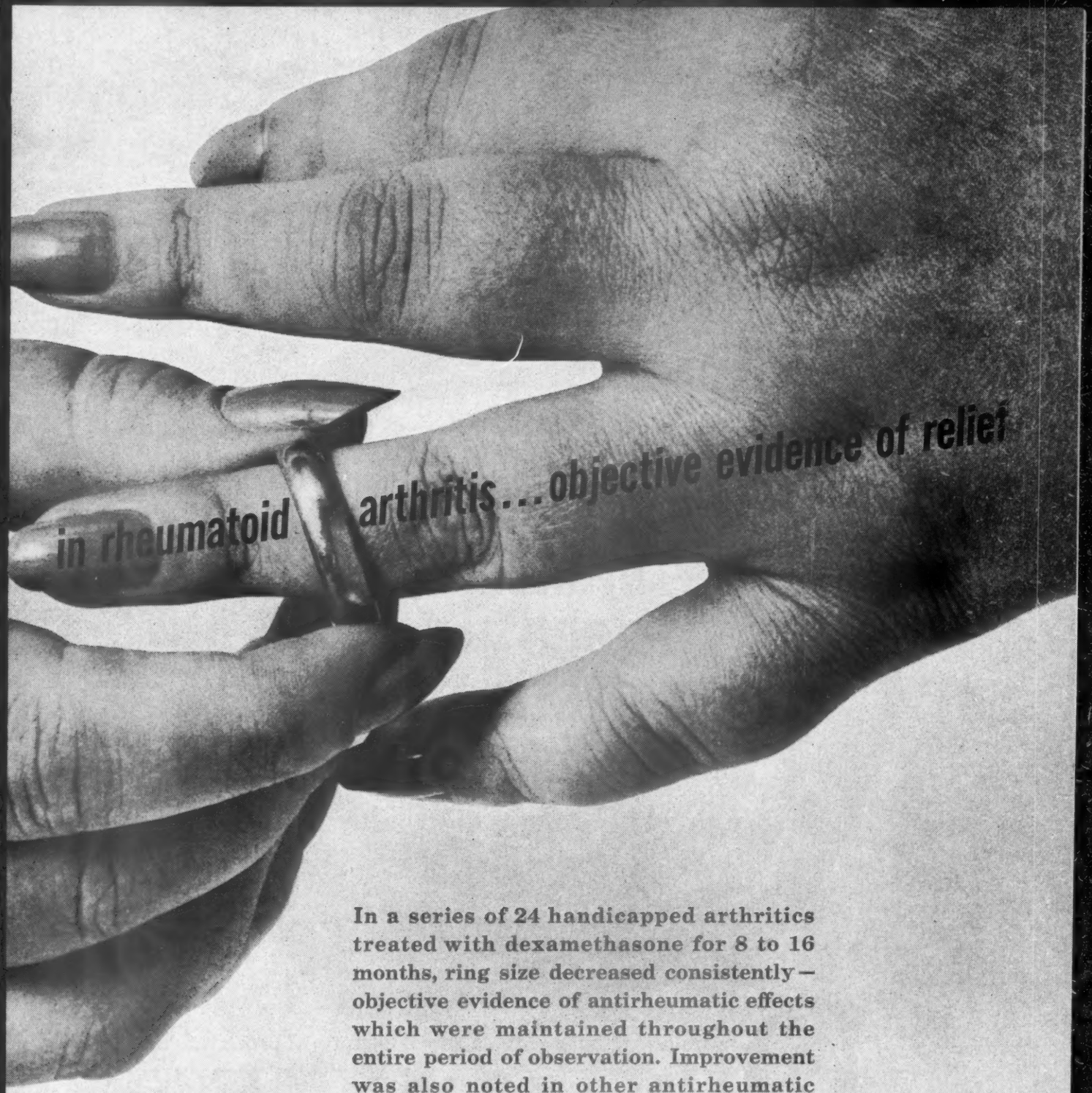

In a series of 24 handicapped arthritics treated with dexamethasone for 8 to 16 months, ring size decreased consistently—objective evidence of antirheumatic effects which were maintained throughout the entire period of observation. Improvement was also noted in other antirheumatic indices, i. e., pain on motion, tenderness, swelling and morning stiffness.[1]

Supplied: as 0.75 mg. and 0.5 mg. scored, pentagon-shaped tablets in bottles of 100. Also available as Injection DECADRON Phosphate and new Elixir DECADRON. Additional information on DECADRON is available to physicians on request. DECADRON is a trademark of Merck & Co., Inc.

Reference: 1. Bunim, J. J., in Hollander, J. L.: Arthritis and Allied Conditions, ed. 6, Philadelphia, Lea & Febiger, 1960, p. 364.

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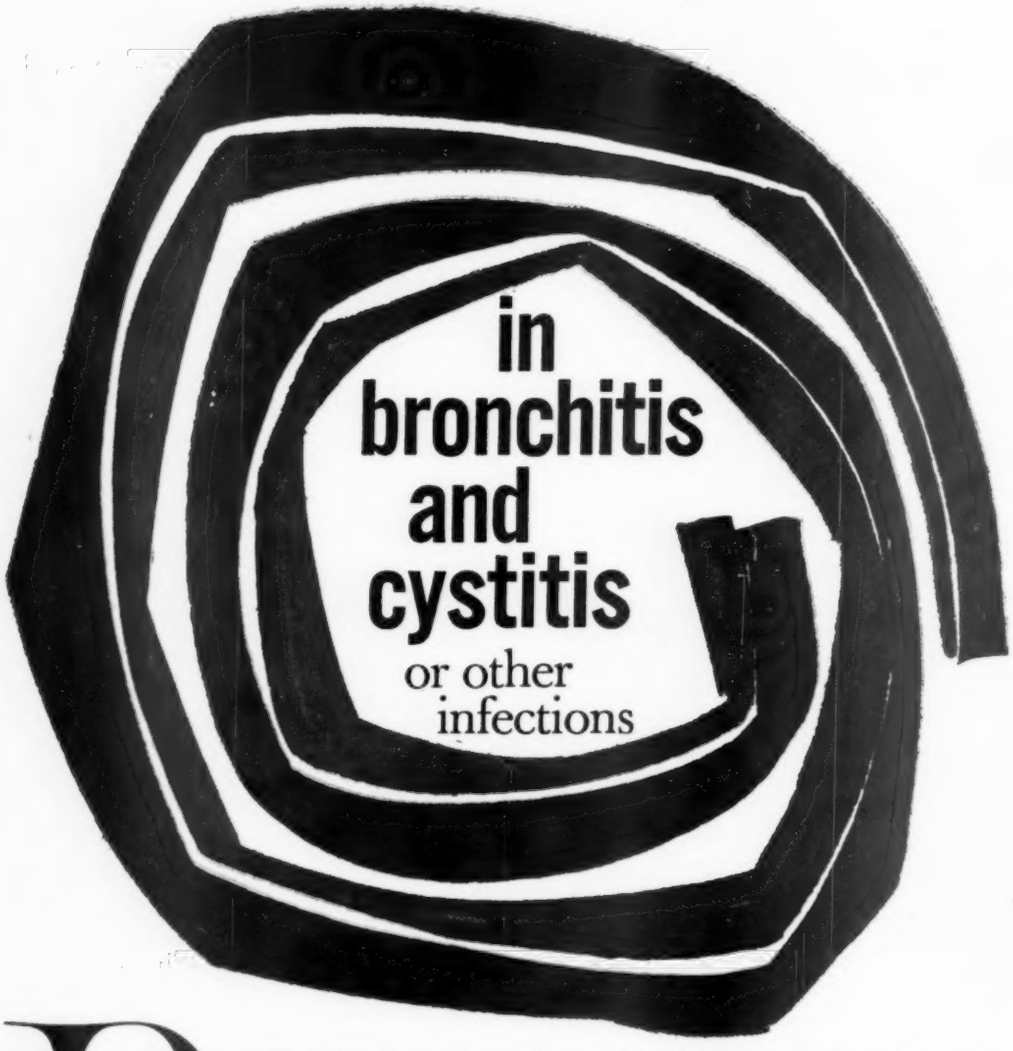
Dexamethasone

TREATS MORE PATIENTS MORE EFFECTIVELY



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against relapse—up to 6 days' activity on 4 days' dosage
against secondary infection—sustained high activity levels
against "problem" pathogens—positive broad-spectrum antibiosis

CAPSULES, 150 mg., 75 mg.; PEDIATRIC DROPS, 60 mg./cc.; SYRUP, 75 mg./5 cc.

Request complete information on indications, dosage, precautions and contraindications from your Lederle representative or write to Medical Advisory Department.

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Often the only therapy needed to control blood pressure and relieve symptoms... potentiates other antihypertensives when used adjunctively ...minimal side effects... economically priced.

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she wakes refreshed

morning dose controls blood pressure all day

her food tastes better
(thanks to salt liberalization)

edema relieved
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"cardiac fears" allayed
(zest for life returns)

Clinically Proven

in more than 750 published clinical studies
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Outstandingly Safe and Effective

for the tense and nervous patient

1 simple dosage schedule relieves anxiety dependably — without the unknown dangers of "new and different" drugs

2 does not produce ataxia, stimulate the appetite or alter sexual function

3 no cumulative effects in long-term therapy

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5 does not muddle the mind or affect normal behavior

Usual dosage: One or two 400 mg. tablets t.i.d.
Supplied: 400 mg. scored tablets, 200 mg. sugar-coated tablets; bottles of 50. Also as MEPROTABS*—400 mg. *unmarked*, coated tablets; and in *sustained-release* capsules as MEPROSPAN®-400 and MEPROSPAN®-200 (containing respectively 400 mg. and 200 mg. meprobamate).
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meprobamate (Wallace)

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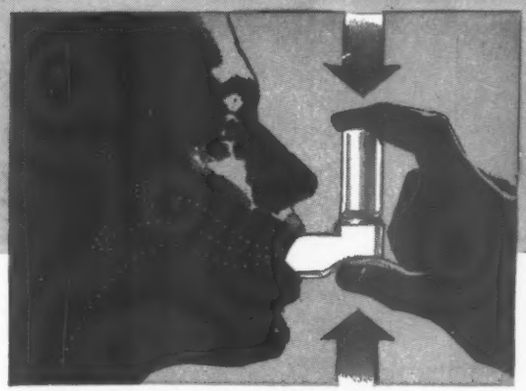

Available with either of the two outstanding bronchodilators

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Isoproterenol sulfate, 2.0 mg. per cc., suspended in inert, nontoxic aerosol vehicle. Contains no alcohol. Each automatically measured dose contains 0.075 mg. isoproterenol.

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For complete information about Forhistal (including dosage, cautions, and side effects), see Physicians' Desk Reference or write CIBA, Summit, N.J.

FORHISTAL® maleate
(dimethpyrindene maleate CIBA)
LONTABS® (long-acting tablets CIBA)

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SUMMIT, NEW JERSEY

Forhistal® rated better than previous therapy in 7 cases out of 10

Forhistal Lontabs® for greater convenience, smoother response, prolonged action.

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